

DESCRIPTION

STAPHYLOCOCCUS AUREUS ANTIBACTERIAL TARGET GENES

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RELATED APPLICATIONS

This application claims priority to Martin et al., *STAPHYLOCOCCUS AUREUS ANTIBACTERIAL TARGET GENES*, United States Provisional Application No. 60/003,798, filed
10 September 15, 1995, and to Benton et al., *STAPHYLOCOCCUS AUREUS ANTIBACTERIAL TARGET GENES*, United States Provisional Application No. 60/009,102, filed December 22, 1995, which are incorporated herein by reference including drawings.

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BACKGROUND

This invention relates to the field of antibacterial treatments and to targets for antibacterial agents. In particular, it relates to genes essential for survival of a bacterial strain *in vitro* or *in vivo*.

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The following background information is not admitted to be prior art to the pending claims, but is provided only to aid the understanding of the reader.

25

Despite the development of numerous antibacterial agents, bacterial infections continue as a major, and currently increasing, medical problem. Prior to the 1980s, bacterial infections in developed countries could be readily treated with available antibiotics. However, during the 1980s and 1990s, antibiotic resistant bacterial strains emerged and have become a major therapeutic problem. There

are, in fact, strains resistant to essentially all of the commonly used antibacterial agents, which have been observed in the clinical setting, notably including strains of *Staphylococcus aureus*. The consequences of the increase in resistant strains include higher morbidity and mortality, longer patient hospitalization, and an increase in treatment costs. (B. Murray, 1994, *New Engl. J. Med.* 330:1229-1230.) Therefore, there is a pressing need for the development of new antibacterial agents which are not significantly affected by the existing bacterial resistance mechanisms.

Such development of new antibacterial agents can proceed by a variety of methods, but generally fall into at least two categories. The first is the traditional approach of screening for antibacterial agents without concern for the specific target.

The second approach involves the identification of new targets, and the subsequent screening of compounds to find antibacterial agents affecting those targets. Such screening can involve any of a variety of methods, including screening for inhibitors of the expression of a gene, or of the product of a gene, or of a pathway requiring that product. However, generally the actual target is a protein, the inhibition of which prevents the growth or pathogenesis of the bacterium. Such protein targets can be identified by identifying genes encoding proteins essential for bacterial growth.

SUMMARY

Each pathogenic bacterial species expresses a number of different genes which are essential for growth of the bacteria in vitro or in vivo in an infection, and which are useful targets for antibacterial agents. This invention provides an approach to the identification of those genes, and the use of those genes, and bacterial strains expressing mutant forms of those genes, in the identification, characterization, and evaluation of targets of antibacterial agents. It further provides the use of those genes and mutant strains in screening for antibacterial agents active against the genes, including against the corresponding products and pathways. Such active compounds can be developed into antibacterial agents. Thus, this invention also provides methods of treating bacterial infections in mammals by administering an antibacterial agent active against such a gene, and the pharmaceutical compositions effective for such treatment.

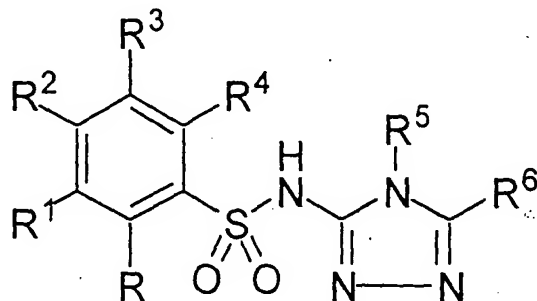
For the *Staphylococcus aureus* essential genes identified in this invention, the essential nature of the genes was determined by the isolation of growth conditional mutants of *Staphylococcus aureus*, in this case temperature sensitive mutants (ts mutants). Each gene was then identified by isolating recombinant bacteria derived from the growth conditional mutant strains, which would grow under non-permissive conditions but which were not revertants. These recombinant bacteria contained DNA inserts derived from the normal (i.e., wild-type) *S. aureus* chromosome which encoded non-mutant products which replaced

the function of the products of the mutated genes. The fact that a clone having such a recombinant insert can complement the mutant gene product under non-permissive conditions implies that the insert contains essentially a complete gene, since it produces functional product.

The *Staphylococcal* genes described herein have either been completely sequenced or have been partially sequenced in a manner which essentially provides the complete gene by uniquely identifying the coding sequence in question, and providing sufficient guidance to obtain the complete sequence and equivalent clones. For example, in some cases, sequences have been provided which can be used to construct PCR primers for amplification of the gene from a genomic sequence or from a cloning vector, e.g., a plasmid. The primers can be transcribed from DNA templates, or preferably synthesized by standard techniques. The PCR process using such primers provides specific amplification of the corresponding gene. Therefore, the complete gene sequence is obtainable by using the sequences provided.

In a first aspect, this invention provides a method of treating a bacterial infection in a mammal by administering a compound which is active against a bacterial gene selected from the group of genes corresponding to SEQ ID NO. 1-105. Each of these genes has been identified as an essential gene by the isolation of growth conditional mutant strains, and the complementation in recombinant strains of each of the mutated genes under non-permissive conditions, by expression from artificially-inserted DNA sequences

carrying genes identified by the specified sequences of SEQ ID NO. 1-105. In particular embodiments of this method, the infection involves a bacterial strain expressing a gene corresponding to one of the specified sequences, or a
 5 homologous gene. Such homologous genes provide equivalent biological function in other bacterial species. Also in a preferred embodiment, the compound has a structure described by the general structure below:



10

in which

R, R¹, R², and R³ are independently H, alkyl (C₁-C₅), or halogen;

15 R⁴ is H, alkyl (C₁-C₅), halogen, SH, or S-alkyl (C₁-C₃);

R⁵ is H, alkyl (C¹-C⁵), or aryl (C₆-C₁₀);

R⁶ is CH₂NH₂, alkyl (C₁-C₄), 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, or aryl (C₆-C₁₀);

20 or

R⁵ and R⁶ together are -C(R⁷)=C(R⁸)-C(R⁹)=C(R¹⁰)-, -N=C(R⁸)-C(R⁹)=C(R¹⁰)-, -C(R⁷)=N-C(R⁹)=C(R¹⁰)-, -C(R⁷)=C(R⁸)-N=C(R¹⁰)-, or -C(R⁷)=C(R⁸)-C(R⁹)=N-;

in which

R^7 , R^8 , R^9 , and R^{10} are independently H, alkyl (C_1-C_5), halogen, fluoroalkyl (C_1-C_5);

or

R^7 and R^8 together are $-CH=CH-CH=CH-$.

5 The term "alkyl" refers to a branched or unbranched aliphatic hydrocarbon group, e.g., methyl, ethyl, *n*-propyl, *iso*-propyl, and *tert*-butyl. Preferably the group includes from 1 to 5 carbon atoms and is unsubstituted, but alternatively may optionally be substituted with functional
10 groups which are commonly attached to such chains, e.g., hydroxyl, fluoro, chloro, aryl, nitro, amino, amido, and the like.

 The term "halogen" refers to a substituent which is fluorine, chlorine, bromine, or iodine. Preferably the
15 substituent is fluorine.

 The term "pyridyl" refers to a group from pyridine, generally having the formula C_5H_4N , forming a heterocyclic ring, which may optionally be substituted with groups commonly attached to such rings.

20 The term furyl refers to a heterocyclic group, having the formula C_4H_3O , which may be either the alpha or beta isomer. The ring may optionally be substituted with groups commonly attached to such rings.

 The term "thienyl" refers to a group from thiophen, generally having a formula C_4H_3S
25

 The term "aryl" refers to an aromatic hydrocarbon group which includes a ring structure in which the electrons are delocalized. Commonly, aryl groups contain a derivative

of the benzene ring. The ring may optionally be substituted with groups commonly attached to aromatic rings, e.g., OH, CH₃, and the like.

The term "fluoroalkyl" refers to an alkyl group, as described above, which one or more hydrogens are substituted with fluorine.

"Treating", in this context, refers to administering a pharmaceutical composition for prophylactic and/or therapeutic purposes. The term "prophylactic treatment" refers to treating a patient who is not yet infected, but who is susceptible to, or otherwise at risk, of a particular infection. The term "therapeutic treatment" refers to administering treatment to a patient already suffering from an infection.

The term "bacterial infection" refers to the invasion of the host mammal by pathogenic bacteria. This includes the excessive growth of bacteria which are normally present in or on the body of a mammal. More generally, a bacterial infection can be any situation in which the presence of a bacterial population(s) is damaging to a host mammal. Thus, a mammal is "suffering" from a bacterial infection when excessive numbers of a bacterial population are present in or on a mammal's body, or when the effects of the presence of a bacterial population(s) is damaging the cells or other tissue of a mammal.

In the context of this disclosure, "bacterial gene" should be understood to refer to a unit of bacterial heredity as found in the chromosome of each bacterium. Each

gene is composed of a linear chain of deoxyribonucleotides which can be referred to by the sequence of nucleotides forming the chain. Thus, "sequence" is used to indicate both the ordered listing of the nucleotides which form the chain, and the chain, itself, which has that sequence of nucleotides. ("Sequence" is used in the same way in referring to RNA chains, linear chains made of ribonucleotides.) The gene includes regulatory and control sequences, sequences which can be transcribed into an RNA molecule, and may contain sequences with unknown function. The majority of the RNA transcription products are messenger RNAs (mRNAs), which include sequences which are translated into polypeptides and may include sequences which are not translated. It should be recognized that small differences in nucleotide sequence for the same gene can exist between different bacterial strains, or even within a particular bacterial strain, without altering the identity of the gene.

Thus, "expressed bacterial gene" means that, in a bacterial cell of interest, the gene is transcribed to form RNA molecules. For those genes which are transcribed into mRNAs, the mRNA is translated to form polypeptides. More generally, in this context, "expressed" means that a gene product is formed at the biological level which would normally have the relevant biological activity (i.e., RNA or polypeptide level).

As used herein in referring to the relationship between a specified nucleotide sequence and a gene, the term "corresponds" or "corresponding" indicates that the

specified sequence identifies the gene. Therefore, a sequence which will uniquely hybridize with a gene from the relevant bacterium corresponds to that gene (and the converse). In general, for this invention, the specified sequences have the same sequence (a low level of sequencing error or individual variation does not matter) as portions of the gene or flanking sequences. Similarly, correspondence is shown by a transcriptional, or reverse transcriptional relationship. Many genes can be transcribed to form mRNA molecules. Therefore, there is a correspondence between the entire DNA sequence of the gene and the mRNA which is, or might be, transcribed from that gene; the correspondence is also present for the reverse relationship, the messenger RNA corresponds with the DNA of the gene. This correspondence is not limited to the relationship between the full sequence of the gene and the full sequence of the mRNA, rather it also exists between a portion or portions of the DNA sequence of the gene and a portion or portions of the RNA sequence of the mRNA. Specifically it should be noted that this correspondence is present between a portion or portions of an mRNA which is not normally translated into polypeptide and all or a portion of the DNA sequence of the gene.

Similarly, the DNA sequence of a gene or the RNA sequence of an mRNA "corresponds" to the polypeptide encoded by that gene and mRNA. This correspondence between the mRNA and the polypeptide is established through the translational relationship; the nucleotide sequence of the mRNA is

translated into the amino acid sequence of the polypeptide.

Then, due to the transcription relationship between the DNA of the gene and the mRNA, there is a "correspondence" between the DNA and the polypeptide.

5 The term "administration" or "administering" refers to a method of giving a dosage of an antibacterial pharmaceutical composition to a mammal, where the method is, e.g., topical, oral, intravenous, transdermal, intraperitoneal, or intramuscular. The preferred method of
10 administration can vary depending on various factors, e.g., the components of the pharmaceutical composition, the site of the potential or actual bacterial infection, the bacterium involved, and the severity of an actual bacterial infection.

15 The term "active against" in the context of compounds, agents, or compositions having antibacterial activity indicates that the compound exerts an effect on a particular bacterial target or targets which is deleterious to the *in vitro* and/or *in vivo* growth of a bacterium having
20 that target or targets. In particular, a compound active against a bacterial gene exerts an action on a target which affects an expression product of that gene. This does not necessarily mean that the compound acts directly on the expression product of the gene, but instead indicates that
25 the compound affects the expression product in a deleterious manner. Thus, the direct target of the compound may be, for example, at an upstream component which reduces transcription from the gene, resulting in a

lower level of expression. Likewise, the compound may affect the level of translation of a polypeptide expression product, or may act on a downstream component of a biochemical pathway in which the expression product of the gene has a major biological role. Consequently, such a compound can be said to be active against the bacterial gene, against the bacterial gene product, or against the related component either upstream or downstream of that gene or expression product. While the term "active against" encompasses a broad range of potential activities, it also implies some degree of specificity of target. Therefore, for example, a general protease is not "active against" a particular bacterial gene which produces a polypeptide product. In contrast, a compound which inhibits a particular enzyme is active against that enzyme and against the bacterial gene which codes for that enzyme.

The term "mammal" refers to any organism of the Class Mammalia of higher vertebrates that nourish their young with milk secreted by mammary glands, e.g., mouse, rat, and, in particular, human, dog, and cat.

By "comprising" it is meant including, but not limited to, whatever follows the word "comprising". Thus, use of the term "comprising" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or

mandatory, and that no other elements may be present. By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements.

Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

A DNA containing a specific bacterial gene is obtainable using a shorter, unique probe(s) with readily available molecular biology techniques. If the method for obtaining such gene is properly performed, it is virtually certain that a longer DNA sequence comprising the desired sequence (such as the full coding sequence or the full length gene sequence) will be obtained. Thus, "obtainable by" means that an isolation process will, with high probability (preferably at least 90%), produce a DNA sequence which includes the desired sequence. Thus, for example, a full coding sequence is obtainable by hybridizing the DNA of two PCR primers appropriately derived from the sequences of SEQ ID NO. 1-105 corresponding to a particular complementing clone to a *Staphylococcus aureus* chromosome, amplifying the sequence between the primers, and purifying the PCR products. The PCR products can then be used for sequencing the entire gene or for other manipulations. Those skilled in the art will understand the included steps,

techniques, and conditions for such processes. However, the full coding sequence or full gene is clearly not limited to a specific process by which the sequence is obtainable. Such a process is only one method of producing the final product.

A "coding sequence" or "coding region" refers to an open reading frame (ORF) which has a base sequence which is normally transcribed in a cell (e.g., a bacterial cell) to form RNA, which in most cases is translated to form a polypeptide. For the genes for which the product is normally a polypeptide, the coding region is that portion which encodes the polypeptide, excluding the portions which encode control and regulatory sequences, such as stop codons and promoter sequences.

In a related aspect, the invention provides a method for treating a bacterial infection in a mammal by administering an amount of an antibacterial agent effective to reduce the infection. The antibacterial agent specifically inhibits a biochemical pathway requiring the expression product of a gene corresponding to one of the genes identified in the first aspect above. Inhibition of that pathway inhibits the growth of the bacteria *in vivo*. In particular embodiments, the antibacterial agent inhibits the expression product of one of the identified genes.

In the context of the coding sequences and genes of this invention, "homologous" refers to genes whose expression results in expression products which have a combination of amino acid sequence similarity (or base

sequence similarity for transcript products) and functional equivalence, and are therefore homologous genes. In general such genes also have a high level of DNA sequence similarity (i.e., greater than 80% when such sequences are identified among members of the same genus, but lower when these similarities are noted across bacterial genera), but are not identical. Relationships across bacterial genera between homologous genes are more easily identified at the polypeptide (i.e., the gene product) rather than the DNA level. The combination of functional equivalence and sequence similarity means that if one gene is useful, e.g., as a target for an antibacterial agent, or for screening for such agents, then the homologous gene is likewise useful. In addition, identification of one such gene serves to identify a homologous gene through the same relationships as indicated above. Typically, such homologous genes are found in other bacterial species, especially, but not restricted to, closely related species. Due to the DNA sequence similarity, homologous genes are often identified by hybridizing with probes from the initially identified gene under hybridizing conditions which allow stable binding under appropriately stringent conditions (e.g., conditions which allow stable binding with approximately 85% sequence identity). The equivalent function of the product is then verified using appropriate biological and/or biochemical assays.

In this context, the term "biochemical pathway" refers to a connected series of biochemical reactions

normally occurring in a cell, or more broadly a cellular event such as cellular division or DNA replication. Typically, the steps in such a biochemical pathway act in a coordinated fashion to produce a specific product or products or to produce some other particular biochemical action. Such a biochemical pathway requires the expression product of a gene if the absence of that expression product either directly or indirectly prevents the completion of one or more steps in that pathway, thereby preventing or significantly reducing the production of one or more normal products or effects of that pathway. Thus, an agent specifically inhibits such a biochemical pathway requiring the expression product of a particular gene if the presence of the agent stops or substantially reduces the completion of the series of steps in that pathway. Such an agent, may, but does not necessarily, act directly on the expression product of that particular gene.

The term "in vivo" in the context of a bacterial infection refers to the host infection environment, as distinguished, for example, from growth of the bacteria in an artificial culture medium (e.g., in vitro).

The term "antibacterial agent" refers to both naturally occurring antibiotics produced by microorganisms to suppress the growth of other microorganisms, and agents synthesized or modified in the laboratory which have either bactericidal or bacteriostatic activity, e.g., β -lactam antibacterial agents, glycopeptides, macrolides, quinolones, tetracyclines, and aminoglycosides. In general, if an

antibacterial agent is bacteriostatic, it means that the agent essentially stops bacterial cell growth (but does not kill the bacteria); if the agent is bacteriocidal, it means that the agent kills the bacterial cells (and may stop growth before killing the bacteria).

The term, "bacterial gene product" or "expression product" is used to refer to a polypeptide or RNA molecule which is encoded in a DNA sequence according to the usual transcription and translation rules, which is normally expressed by a bacterium. Thus, the term does not refer to the translation of a DNA sequence which is not normally translated in a bacterial cell. However, it should be understood that the term does include the translation product of a portion of a complete coding sequence and the translation product of a sequence which combines a sequence which is normally translated in bacterial cells translationally linked with another DNA sequence. The gene product can be derived from chromosomal or extrachromosomal DNA, or even produced in an *in vitro* reaction. Thus, as used herein, an "expression product" is a product with a relevant biological activity resulting from the transcription, and usually also translation, of a bacterial gene.

In another related aspect, the invention provides a method of inhibiting the growth of a pathogenic bacterium by contacting the bacterium with an antibacterial agent which specifically inhibits a biochemical pathway requiring the expression product of a gene selected from the group of

genes corresponding to SEQ ID NO. 1-105 or a homologous gene. Inhibition of that pathway inhibits growth of the bacterium. In particular embodiments, the antibacterial agent inhibits the expression product of one of the
5 identified genes. Also in preferred embodiment, the antibacterial agent is a compound having a structure as described in the first aspect above.

The term "inhibiting the growth" indicates that the rate of increase in the numbers of a population of a particular bacterium is reduced. Thus, the term includes
10 situations in which the bacterial population increases but at a reduced rate, as well as situations where the growth of the population is stopped, as well as situations where the numbers of the bacteria in the population are reduced
15 or the population even eliminated.

A "pathogenic bacterium" includes any bacterium capable of infecting and damaging a mammalian host, and, in particular, includes *Staphylococcus aureus*. Thus, the term includes both virulent pathogens which, for example, can
20 cause disease in a previously healthy host, and opportunistic pathogens which can only cause disease in a weakened or otherwise compromised host.

Similarly, the invention provides a method of prophylactic treatment of a mammal by administering a
25 compound active against a gene selected from the group of genes corresponding to SEQ ID NO. 1-105 to a mammal at risk of a bacterial infection.

A mammal may be at risk of a bacterial infection, for example, if the mammal is more susceptible to infection or if the mammal is in an environment in which infection by one or more bacteria is more likely than in a normal setting. Therefore, such treatment can, for example, be appropriate for an immuno-compromised patient.

Also provided is a method of screening for an antibacterial agent by determining whether a test compound is active against one of the genes identified in the first aspect. In a particular embodiment the method is performed by providing a bacterial strain having a mutant form of a gene selected from the group of genes corresponding to SEQ. ID. NOS. 1-105 or a mutant gene homologous to one of those genes. The mutant form of the gene confers a growth conditional phenotype, e.g., a temperature-sensitive phenotype, on the bacterial strain having that mutant form.

A comparison bacterial strain having a normal form of the gene is also provided and the two strains of bacteria are separately contacted with a test compound under semi-permissive growth conditions. The growth of the two strains in the presence of the test compound is then compared; a reduction in the growth of the bacterial strain having the mutant form compared to the growth of the bacterial strain having the normal form of the gene indicates that the test compound is active against the particular gene.

In this context, a "mutant form" of a gene is a gene which has been altered, either naturally or

artificially, changing the base sequence of the gene, which results in a change in the amino acid sequence of an encoded polypeptide. The change in the base sequence may be of several different types, including changes of one or more bases for different bases, small deletions, and small insertions. By contrast, a normal form of a gene is a form commonly found in a natural population of a bacterial strain. Commonly a single form of a gene will predominate in natural populations. In general, such a gene is suitable as a normal form of a gene, however, other forms which provide similar functional characteristics may also be used as a normal gene. In particular, a normal form of a gene does not confer a growth conditional phenotype on the bacterial strain having that gene, while a mutant form of a gene suitable for use in these methods does provide such a growth conditional phenotype.

As used in this disclosure, the term "growth conditional phenotype" indicates that a bacterial strain having such a phenotype exhibits a significantly greater difference in growth rates in response to a change in one or more of the culture parameters than an otherwise similar strain not having a growth conditional phenotype. Typically, a growth conditional phenotype is described with respect to a single growth culture parameter, such as temperature. Thus, a temperature (or heat-sensitive) mutant (i.e., a bacterial strain having a heat-sensitive phenotype) exhibits significantly reduced growth, and preferably no growth, under non-permissive temperature

conditions as compared to growth under permissive conditions. In addition, such mutants preferably also show intermediate growth rates at intermediate, or semi-permissive, temperatures. Similar responses also result
5 from the appropriate growth changes for other types of growth conditional phenotypes.

Thus, "semi-permissive conditions" are conditions in which the relevant culture parameter for a particular growth conditional phenotype is intermediate between
10 permissive conditions and non-permissive conditions. Consequently, in semi-permissive conditions the bacteria having a growth conditional phenotype will exhibit growth rates intermediate between those shown in permissive conditions and non-permissive conditions. In general, such
15 intermediate growth rate is due to a mutant cellular component which is partially functional under semi-permissive conditions, essentially fully functional under permissive conditions, and is non-functional or has very low function under non-permissive conditions, where the
20 level of function of that component is related to the growth rate of the bacteria.

The term "method of screening" means that the method is suitable, and is typically used, for testing for a particular property or effect in a large number of
25 compounds. Therefore, the method requires only a small amount of time for each compound tested; typically more than one compound is tested simultaneously (as in a 96-well microtiter plate), and preferably significant portions of

the procedure can be automated. "Method of screening" also refers to determining a set of different properties or effects of one compound simultaneously.

Since the essential genes identified herein can be readily isolated and the gene products expressed by routine methods, the invention also provides the polypeptides encoded by those genes. Thus, the invention provides a method of screening for an antibacterial agent by determining the effects of a test compound on the amount or level of activity of a polypeptide gene product of one of the identified essential genes. The method involves contacting cells expressing such a polypeptide with a test compound, and determining whether the test compound alters the amount or level of activity of the expression product. The exact determination method will be expected to vary depending on the characteristics of the expression product.

Such methods can include, for example, antibody binding methods, enzymatic activity determinations, and substrate analog binding assays.

It is quite common in identifying antibacterial agents, to assay for binding of a compound to a particular polypeptide where binding is an indication of a compound which is active to modulate the activity of the polypeptide.

Thus, by identifying certain essential genes, this invention provides a method of screening for an antibacterial agent by contacting a polypeptide encoded by one of the identified essential genes, or a biologically active fragment of such a polypeptide, with a test compound,

and determining whether the test compound binds to the polypeptide or polypeptide fragment.

In addition, to simple binding determinations, the invention provides a method for identifying or evaluating an agent active on one of the identified essential genes. The method involves contacting a sample containing an expression product of one of the identified genes with the known or potential agent, and determining the amount or level of activity of the expression product in the sample.

In a further aspect, this invention provides a method of diagnosing the presence of a bacterial strain having one of the genes identified above, by probing with an oligonucleotide at least 15 nucleotides in length, which specifically hybridizes to a nucleotide sequence which is the same as or complementary to the sequence of one of the bacterial genes identified above. In some cases, it is practical to detect the presence of a particular bacterial strain by direct hybridization of a labeled oligonucleotide to the particular gene. In other cases, it is preferable to first amplify the gene or a portion of the gene before hybridizing labeled oligonucleotides to those amplified copies.

In a related aspect, this invention provides a method of diagnosing the presence of a bacterial strain by specifically detecting the presence of the transcriptional or translational product of the gene. Typically, a transcriptional (RNA) product is detected by hybridizing a labeled RNA or DNA probe to the transcript. Detection of a

specific translational (protein) product can be performed by a variety of different tests depending on the specific protein product. Examples would be binding of the product by specific labeled antibodies and, in some cases, detection
5 of a specific reaction involving the protein product.

As used above and throughout this application, "hybridize" has its usual meaning from molecular biology. It refers to the formation of a base-paired interaction between nucleotide polymers. The presence of base pairing
10 implies that at least an appreciable fraction of the nucleotides in each of two nucleotide sequences are complementary to the other according to the usual base pairing rules. The exact fraction of the nucleotides which must be complementary in order to obtain stable
15 hybridization will vary with a number of factors, including nucleotide sequence, salt concentration of the solution, temperature, and pH.

The term, "DNA molecule", should be understood to refer to a linear polymer of deoxyribonucleotides, as well
20 as to the linear polymer, base-paired with its complementary strand, forming double-strand DNA (dsDNA). The term is used as equivalent to "DNA chain" or "a DNA" or "DNA polymer" or "DNA sequence":, so this description of the term meaning applies to those terms also. The term does not necessarily
25 imply that the specified "DNA molecule" is a discrete entity with no bonding with other entities. The specified DNA molecule may have H-bonding interactions with other DNA molecules, as well as a variety of interactions with other

molecules, including RNA molecules. In addition, the specified DNA molecule may be covalently linked in a longer DNA chain at one, or both ends. Any such DNA molecule can be identified in a variety of ways, including, by its particular nucleotide sequence, by its ability to base pair under stringent conditions with another DNA or RNA molecule having a specified sequence, or by a method of isolation which includes hybridization under stringent conditions with another DNA or RNA molecule having a specified sequence.

References to a "portion" of a DNA or RNA chain mean a linear chain which has a nucleotide sequence which is the same as a sequential subset of the sequence of the chain to which the portion refers. Such a subset may contain all of the sequence of the primary chain or may contain only a shorter sequence. The subset will contain at least 15 bases in a single strand.

However, by "same" is meant "substantially the same"; deletions, additions, or substitutions of specific nucleotides of the sequence, or a combination of these changes, which affect a small percentage of the full sequence will still leave the sequences substantially the same. Preferably this percentage of change will be less than 20%, more preferably less than 10%, and even more preferably less than 3%. "Same" is therefore distinguished from "identical"; for identical sequences there cannot be any difference in nucleotide sequences.

As used in reference to nucleotide sequences, "complementary" has its usual meaning from molecular

biology. Two nucleotide sequences or strands are complementary if they have sequences which would allow base pairing between the strands according to the usual pairing rules. This does not require that the strands would necessarily base pair at every nucleotide; two sequences can still be complementary with a low level of base mismatch such as that created by deletion, addition, or substitution of one or a few (up to 5 in a linear chain of 25 bases) nucleotides, or a combination of such changes.

Further, in another aspect, this invention provides a pharmaceutical composition appropriate for use in the methods of treating bacterial infections described above, containing a compound active on a bacterial gene selected from the group of genes described above and a pharmaceutically acceptable carrier. In a preferred embodiment, the compound has a structure as described in the first aspect above. Also, in a related aspect the invention provides a novel compound having antibacterial activity against one of the bacterial genes described above.

In a further related aspect a method of making an antibacterial agent is provided. The method involves screening for an agent active on one of the identified essential genes by providing a bacterial strain having a mutant form of one of the genes corresponding to SEQ ID NO. 1-105, or a homologous gene. As described above, the mutant form of the gene confers a growth conditional phenotype. A comparison bacterial strain is provided which has a normal form of said gene. The bacterial strains are contacted with

a test compound in semi-permissive growth conditions, and the growth of the strains are compared to identify an antibacterial agent. The identified agent is synthesized in an amount sufficient to provide the agent in a therapeutically effective amount to a patient.

A "carrier" or "excipient" is a compound or material used to facilitate administration of the compound, for example, to increase the solubility of the compound. Solid carriers include, e.g., starch, lactose, dicalcium phosphate, sucrose, and kaolin. Liquid carriers include, e.g., sterile water, saline, buffers, non-ionic surfactants, and edible oils such as peanut and sesame oils. In addition, various adjuvants such as are commonly used in the art may be included. These and other such compounds are described in the literature, e.g., in the *Merck Index*, Merck & Company, Rahway, NJ. Considerations for the inclusion of various components in pharmaceutical compositions are described, e.g., in Gilman et al. (Eds.) (1990); Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 8th Ed., Pergamon Press.

Consistent with the usage of "anti-bacterial agent" herein, the term "anti-bacterial activity" indicates that the presence of a particular compound in the growth environment of a bacterial population reduces the growth rate of that population, without being a broad cellular toxin for other categories of cells.

As is described below in the Detailed Description of the Preferred Embodiments, bacterial strains expressing a

mutated form of one of the above identified genes, which confers a growth conditional phenotype, are useful for evaluating and characterizing the gene as an antibacterial target and for screening for antibacterial agents.

- 5 Therefore, this invention also provides a purified bacterial strain expressing a mutated gene which is a mutated form of one of the bacterial genes identified above, where the mutated gene confers a growth conditional phenotype.

Similarly, this invention provides a recombinant
10 bacterial cell containing an artificially inserted DNA construct which contains a DNA sequence which is the same as or complementary to one of the above-identified bacterial genes or a portion of one of those genes. Such cells are useful, for example, as sources of probe sequences or for
15 providing a complementation standard for use in screening methods.

The term "recombinant bacterial cell" has its usual molecular biological meaning. The term refers to a microbe into which has been inserted, through the actions
20 of a person, a DNA sequence or construct which was not previously found in that cell, or which has been inserted at a different location within the cell, or at a different location in the chromosome of that cell. Such a term does not include natural genetic exchange, such as conjugation
25 between naturally occurring organisms. Thus, for example, a recombinant bacterium could have a DNA sequence inserted which was obtained from a different bacterial species, or

may contain an inserted DNA sequence which is an altered form of a sequence normally found in that bacteria.

As described above, the presence of a specific bacterial strain can be identified using oligonucleotide probes. Therefore this invention also provides such oligonucleotide probes at least 15 nucleotides in length, which specifically hybridize to a nucleotide sequence which is the same as or complementary to a portion of one of the bacterial chains identified above.

In a related aspect this invention provides an isolated or purified DNA sequence at least 15 nucleotides in length, which has a nucleotide base sequence which is the same as or complementary to a portion of one of the above-identified bacterial genes. In particular embodiments, the DNA sequence is the same as or complementary to the base sequence of the entire coding region of one of the above-identified bacterial genes. Such an embodiment may in addition contain the control and regulatory sequence associated with the coding sequence.

Use of the term "isolated" indicates that a naturally occurring material or organism (e.g., a DNA sequence) has been removed from its normal environment. Thus, an isolated DNA sequence has been removed from its usual cellular environment, and may, for example, be in a cell-free solution or placed in a different cellular environment. For a molecule, such as a DNA sequence, the term does not imply that the molecule (sequence) is the only molecule of that type present.

It is also advantageous for some purposes that an organism or molecule (e.g., a nucleotide sequence) be in purified form. The term "purified" does not require absolute purity; instead, it indicates that the sequence, 5 organism, or molecule is relatively purer than in the natural environment. Thus, the claimed DNA could not be obtained directly from total human DNA or from total human RNA. The claimed DNA sequences are not naturally occurring, but rather are obtained via manipulation of a partially 10 purified naturally occurring substance (genomic DNA clones).

The construction of a genomic library from chromosomal DNA involves the creation of vectors with genomic DNA inserts and pure individual clones carrying such vectors can be isolated from the library by clonal selection of the cells 15 carrying the library.

In a further aspect, this invention provides an isolated or purified DNA sequence which is the same as or complementary to a bacterial gene homologous to one of the above-identified bacterial genes where the function of the 20 expression product of the homologous gene is the same as the function of the product of one of the above-identified genes. In general, such a homologous gene will have a high level of nucleotide sequence similarity and, in addition, a protein product of homologous gene will have a significant 25 level of amino acid sequence similarity. However, in addition, the product of the homologous gene has the same biological function as the product of the corresponding gene identified above.

Similarly, the invention provides an isolated or purified DNA sequence which has a base sequence which is the same as the base sequence of a mutated bacterial gene selected from one of the genes identified in the first aspect where the expression of this DNA sequence or the mutated bacterial gene confers a growth conditional phenotype in the absence of expression of a gene which complements that mutation. Such an isolated or purified DNA sequence can have the base sequence which varies slightly from the base sequence of the original mutated gene but must contain a base sequence change or changes which are functionally equivalent to the base sequence change or changes in the mutated gene. In most cases, this will mean that the DNA sequence has the identical bases at the site of the mutation as the mutated gene.

As indicated above, by providing the identified essential genes, the encoded expression products are also provided. Thus, another aspect concerns a purified, enriched, or isolated polypeptide, which is encoded by one of the identified essential genes. Such a polypeptide may include the entire gene product or only a portion or fragment of the encoded product. Such fragments are preferably biologically active fragments which retain one or more of the relevant biological activities of the full size gene product.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments, and from the claims.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows the fold increase in sensitivity toward 12 antibacterial agents and a generally toxic agent for 3 temperature sensitive mutants of *Salmonella typhimurium*. These are mutants of DNA gyrase subunit A (*gyrA212*, *gyrA215*, and *gyrA216*, grown at a semi-permissive temperature (35_C). Hypersensitivity is observed to antibacterial agents acting on DNA gyrase, but not to other classes of drugs or toxic agents. The data demonstrate that growth conditional mutations in a known target cause hypersensitivity to target inhibitors.

Fig. 2 presents the hypersensitivity profiles of a set of temperature sensitive mutants of *Salmonella*, for a variety of antibacterial agents with characterized modes of action, compared to the sensitivity profile of wild type.

Fig. 3 illustrates a variety of types of interactions which exist between different essential genes, and which can create differential responses in screens using growth conditional mutants.

Fig. 4 illustrates a possible arrangement of a multichannel screen plate using conditional growth mutants with mutations affecting 5 different cellular processes plus controls.

Fig. 5 illustrates 2 alternative multichannel screen designs in which either multiple compounds are screened using a single growth conditional mutant on each plate, or in which multiple growth conditional mutants are

used on each plate to create an inhibition profile of a single compound.

Fig. 6 is a bar graph showing the different heat sensitivity profiles for 6 *S. aureus* heat sensitive mutant strains. The growth of each strain is shown at 6 different temperatures ranging from 30°C to 43°C.

Fig. 7 is a bar graph showing the different heat sensitivity profiles for 4 different *S. aureus* polC heat sensitive mutants and a wild type strain. The growth of each strain is shown at 6 different temperatures ranging from 30°C to 43°C.

Fig. 8 is a graph showing the differences in hypersensitivity of one *S. aureus* heat sensitive strain (NT99) toward 30 inhibitory compounds at 3 different temperatures.

Fig. 9 is a diagram for two *S. aureus* mutants, illustrating that a greater number of growth inhibitory hits are identified at higher temperatures using heat sensitive mutants. Compounds were identified as hits if the growth of the mutant was inhibited by at least 50% and the inhibition of growth of the mutant was at least 30% higher than the inhibition of growth of a wild type strain.

Fig. 10 is a bar diagram illustrating the effect of test compound concentration on the number of hits identified, showing that, in general, more compounds are identified as hits at higher concentrations.

Fig. 11 presents the structures of two compounds which exhibited the same inhibition profiles for a set of

temperature sensitive *Staphylococcus aureus* mutants, showing the structural similarity of the compounds.

Fig. 12 presents the fold increase in sensitivity of a set of *Staphylococcus aureus* temperature sensitive mutants for a variety of compounds which inhibit growth of *Staphylococcus aureus* wild type, but which have uncharacterized targets of action.

Fig. 13 illustrates the types of anticipated inhibition profiles of different growth conditional mutants for a variety of test compounds, indicating that the number of mutants affected by a particular compound is expected to vary.

Fig. 14 shows the proportion of compounds (from a total of 65) which significantly inhibited the growth of varying numbers of temperature sensitive mutants in a screen of uncharacterized growth inhibitors of *Staphylococcus aureus*.

Fig. 15 shows the potency (MIC values) of a number of growth inhibitors which affected 0, 1 or more than 3 temperature sensitive mutants of *Staphylococcus aureus* in a screen of uncharacterized growth inhibitors.

Fig. 16 shows the number of hits for each of the temperature sensitive mutants of *Staphylococcus aureus* in a screen of 65 uncharacterized growth inhibitors.

Fig. 17 shows some advantages of a multichannel genetic potentiation screen using growth conditional mutants over traditional biochemical screens with either a known target or an unknown cloned gene.

Fig. 18 illustrates a strategy for selecting dominant lethal mutants for use in screens for antibacterial agents, not requiring hypersensitivity.

Fig. 19A-D are structures of four compounds which
5 were identified as hits on mutant NT94.

Fig. 20 is a partial restriction map of the *S. aureus* clone insert (complementing mutant NT64), showing the position of the initial left and right sequences obtained.

Figs. 21-90 are partial restriction maps of each
10 of the *S. aureus* clone inserts for which sequences are described herein, showing the relative fraction of the insert for which nucleotide sequence is described, as well as the approximate positions of identified open reading frames (ORFs).

15

DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. General Approach for Identification of Target Genes

As was briefly described in the Summary above, this invention concerns essential genes in *Staphylococcus aureus*. This organism is a serious pathogen which
20 frequently carries resistance to a variety of existing antibiotic agents. Such resistant strains of *S. aureus* are a particular problem in settings where antibacterial agents are intensively used, such as in hospitals. To overcome the
25 therapeutic difficulties posed by the existing resistant strains, it is highly desirable that new classes of antibiotic drugs be found, particularly ones which are active against new bacterial targets. While such bacterial

targets are usually (though not always) proteins, the targets can be identified by first identifying the bacterial genes which encode proteins (or RNA transcripts) that are essential for growth of the bacteria.

5 Identification of these genes which are essential for growth of the bacteria was accomplished by isolating conditional lethal mutant strains. Such mutant strains will grow under permissive conditions, but will not grow, or grow very poorly under non-permissive conditions. For the
10 bacterial genes described herein, temperature sensitive mutants provided the growth conditional phenotype. The particular gene in each strain which was mutated to confer a growth conditional phenotype was then identified by isolating recombinant derivatives of the mutant strains.
15 These recombinant strains each contained a DNA insert which, when expressed, would complement the defective gene and thus would allow growth under non-permissive conditions. These DNA inserts were provided by a genomic library of a normal *S. aureus* chromosome. The ability of the DNA insert in the
20 recombinant strain to complement the defective product of the mutated gene showed that the DNA insert contained essentially a complete gene corresponding to a particular mutated gene. The vectors carrying each of these DNA
25 inserts were constructed such that the *S. aureus* chromosomal insert could be amplified by PCR using flanking primer sequences. Each of the amplified *S. aureus* inserts was then partially sequenced, in general from both the 5' and 3' ends. This sequencing was, in general, single pass

sequencing and, thus, the specified sequences may contain a low level of sequence errors compared to the actual gene sequence. Since the partial sequences at the 5' and 3' ends bracket the complete gene, such partial sequences uniquely
5 identify and provide that complete gene without interference from a low level of sequencing error. The complete gene and gene sequence can be reliably obtained by any of several different methods. For example, probes can be constructed based on the partial sequences provided, which can be used
10 to probe genomic or cDNA libraries of *S. aureus*. Clones containing the corresponding 5' and 3' sequences can then be further characterized and sequenced to provide the complete gene. In another approach, the partial 5' and 3' sequences can be used to construct PCR primer sequences which can be
15 used to amplify the sequence between those primers and likewise provide the complete gene. In yet another approach, equivalent growth conditional mutant strains can be obtained by following the same or a similar process of mutagenizing the base *S. aureus* strain, and then likewise
20 obtaining the complete gene by isolating complementing clones which correspond to the sequences provided, from a genomic or cDNA library. It should again be noted that, for any of these approaches, a low level of sequencing error in the sequence presented herein does not matter, since the
25 stringency of the hybridizing conditions can be readily adjusted to provide the appropriately specific binding. While the genes identified in this invention are highly useful as targets for novel antibacterial therapy, the genes

and parts of those genes are also useful to provide probes which can be used to identify the presence of a particular bacteria carrying a particular gene. In addition, the growth conditional mutant strains described above are also
5 useful as tools in methods for screening for antibacterial agents which target that gene (targeting the corresponding normal gene). The methods involved in the identification of the mutant strains complementing recombinant clones and the particular genes are described in more detail below.

10 A. Bacterial strain selection

The growth conditional mutant strains and recombinant strains herein are based on *S. aureus* strain 8325-4. This strain has been the subject of substantial genetic characterization and is appropriate for use in the
15 approach described herein. It is believed to be free of transposons, phage or extrachromosomal elements. Numerous other strains of *S. aureus* can likewise be used. However, it is advantageous to select a strain which has few, or preferably no, transposons or extrachromosomal elements, as
20 such elements can complicate the genetic analysis.

B. Isolation of conditional lethal mutants (general).

Heat-sensitive mutants were obtained after diethyl sulfate (DES; SIGMA Chemical) mutagenesis of strain 8325-4.

Briefly, single colonies were inoculated into LB broth in
25 individual wells of a 96-well microtiter plate and grown overnight (35°C, 18 h). Culture supernatants (10 μ l) were diluted into λ -dilution buffer (λ dil; 500 μ l) and then treated with DES (5 μ l). After a short incubation period

(20 min at 37°C), the treated cultures were serially diluted with λ dil into microtiter plates. After an additional incubation period (8-12 h. at 37°C), appropriate dilutions (50 μ l each of 10 E-2 and 10 E-3) were plated onto TS agar plates and incubated overnight (30°C, 18 h). The plates were replica-printed onto two Tryptic-soy (TS) plates and incubated either at 30°C or 43°C (permissive and non-permissive conditions, respectively). Colonies growing at 30°C but not at 43°C were isolated and their ts phenotype was subsequently confirmed in a second round of plating. Only one ts mutant was picked from an original single-colony culture to assure that the mutants isolated were independent from each other. Independently-derived colonies with the appropriate phenotype are identified by direct screening on rich solid media at a permissive temperature (30°C), as it obviates selection of mutants deficient in metabolic pathways, such as aromatic amino acid biosynthesis. No penicillin enrichment is employed, as it would counterselect mutant strains that are strongly bactericidal at the non-permissive temperature. A preliminary collection of 100 independent condition-lethal mutants and 71 non-independent mutants was made. This collection has been supplemented with additional condition-lethal mutants.

C. Creation of the *S. aureus* shuttle library

The *S. aureus* strain used for the preparation of genomic DNA for library construction as well as for the generation of conditional-lethal (temperature sensitive) mutants described in this document is a derivative of NCTC

8325, designated as 8325-4 (Novick, R.P., 1990). The 8325 parent strain is one of the better-characterized strains of *S. aureus*, with genetic and physical map data available in the current literature (Pattee, P.A., 1990). The 8325-4 derivative strain has all the chromosomal elements of the parent, with the exception of integrated (i.e., prophage and transposon DNA) and extrachromosomal (i.e., plasmid DNA) elements endogenous to the parent.

Cloning and subcloning experiments utilized the commercially-available *E. coli* strains JM109 (Promega) and DH5alpha (GIBCO-BRL). All enzymes cited (i.e., restriction endonucleases, ligases and phosphatases) were obtained commercially (NEB, Promega). All DNA cloning and manipulations are described in the current literature (Sambrook, et al., 1989). Parent plasmids pE194 and pUC19 have been described previously (Horinouchi, S. et al., 1982; Yanisch-Perron, C. et al., 1985). Recombinant constructs for use in a *S. aureus* host were first electroporated (Gene Pulser, BioRad) into *S. aureus* strain RN4220 (a restriction-deficient but methylase-proficient strain; Novick, R.P., 1990) before transduction into the target strain for complementation and cross-complementation analyses.

D. Library Construction

The shuttle plasmid vector used was pMP16, constructed by cloning the entire length of the natural *S. aureus* plasmid pE194 (linearized with Cla I) into the Nar I site of pUC19 (Yanisch-Perron et al., 1985). This new construct replicates and offers antibiotic resistance

selections in both *E. coli* and *S. aureus*. It also provides blue-white screening to facilitate scoring of insert-containing clones. Carefully purified genomic DNA from *S. aureus* strain 8325-4 was partially digested (Sau3A I) and fragments of 2-8 kb were isolated by sucrose gradient centrifugation. DNA fragments isolated in this manner were then used for constructing two different libraries. In library A, the DNA fragments were directly cloned into pMP16, which had been linearized (Bam HI) and dephosphorylated (CIP). The DNA mixture was ligated (T4 DNA ligase) and transformed into *E. coli* DH5alpha. Library A thus constructed contains about 60,000 independent clones, 60% of which have inserts. In constructing library B, the ends of the Sau3A I fragments were partially filled with dGTP and dATP, ligated with linearized (Sal I) pMP16 that was partially filled with dCTP and dTTP, and transformed into *E. coli*. The advantage of partially filling the ends is that DNAs with the same ends can no longer ligate to each other; the majority of the ligation occurs between the vector and inserts, significantly increasing the percentage of insert-containing clones. In addition, the chance that two unrelated insert fragment are fortuitously ligated in the same clone is greatly reduced by using this strategy. Library B consists of 50,000 independent clones with > 98% containing inserts. Both library A and library B contain at least a 50-fold representation of the *S. aureus* genome.

Clones from the two libraries were pooled and plasmid DNA extracted. The DNAs were used to transform *S.*

aureus strain RN4220. About 100,000 erythromycin resistant transformants were pooled and infected with bacteriophage ϕ 11 at a multiplicity of infection (MOI) of 0.01 to generate phage lysates containing the shuttle library plasmids. The
5 lysates were then used to introduce the shuttle plasmids into ts mutants by transduction to isolate complementing clones.

E. Isolation of complementing clones (general)

The lysate from library B was first chosen for
10 transduction of the ts mutants because of its higher insert frequency. The ts mutants were grown either in TS broth or on TS agar plates overnight (18 h). The cells were resuspended in TS broth containing CaCl_2 (5 mM) to an OD_{600} between 2 - 3. The lysate from library B (10-50 μ l) was
15 added to the resuspended cells (2 ml) and incubated at 30°C with slow shaking (20 m). Ice-cold sodium citrate (20 mM; 1 ml) was added and the culture was centrifuged to pellet the cells. After removing the supernatant, the pellet was resuspended in ice-cold sodium citrate (20 mM; 500 μ l). A
20 small aliquot (about 1/5000 of the total volume) was plated on a TSA-ery-citrate plate (TS agar containing 5 μ g/ml erythromycin and 500 μ g/ml sodium citrate) and incubated at 30°C overnight (18 h). The total number of erythromycin-resistant transductants screened were estimated
25 from this plate; at least 200,000 transductants were screened for each ts mutant to assure that the library population was well represented. The rest of the cells were plated onto the same selection media (3-5 plates), incubated

at 30°C for 5 h and then at 43°C overnight (18 h). Individual colonies that appeared on the 43°C plates were isolated and infected with ϕ 11 to generate lysates.

The lysates prepared from these individual colonies were then used to transduce the same ts mutants as described above, using much smaller volumes of cells (0.1 ml) and lysates (1-3 μ l) to facilitate testing of large number of lysates. Equal amounts of the transduced cultures were plated onto two sets of TSA-ery-citrate plates and incubated at either 30 or 43°C. Individual lysates that generated similar numbers of transductants at 30 and 43°C were scored as complementing clones. Among the first 96 ts mutants studied, complementing clones were isolated for 60 (63%) of the mutants; 57 were from library B and 3 were from library A.

To test whether different ts mutants carry mutations in the same or closely linked genes, cross complementation was performed to evaluate the ability of positive clones of one ts mutant to complement another mutant. The results showed that, while some positive clones failed to complement any ts mutants other than their primary mutant, other clones were able to complement additional mutants. Taken together, the cross complementation studies identified 38 loci on the *S. aureus* chromosome, each consisting of at least one essential gene.

All the positive clones for the 60 ts mutants were twice streaked on TSA-ery-citrate plates and grown at 43°C to eliminate ϕ 11 prophage from the host cells. Plasmid DNA

was extracted from these complementing clones and transformed into *E. coli*. The plasmids were prepared from the *E. coli* clones and used for restriction mapping and subcloning of the inserts.

5 F. Strategy for DNA sequencing of complementing clones (general)

Complementing clones were subcloned into a sequencing vector (pGEM3Zf(+); Promega) containing regions of DNA flanking the multiple cloning site (T7 and SP6 primer
10 annealing sites) to facilitate plasmid-based automated sequencing. Clones larger than 1.54 kB were cut with restriction endonucleases (BamHI, HindIII, EcoRI; NEB) and then subcloned into the same sequencing vector. DNA sequence ladders were generated by thermocycle sequencing
15 procedures based upon the use of fluorescent-labeled primers (one of T7, SP6, M13 forward and M13 reverse; ABI), a thermostable DNA polymerase (AmpliTag; Perkin Elmer/ABI) and dideoxy terminator chemistry (Sanger, et al, 1977, *Proc. Natl. Acad. Sci. USA* 74:54463). Data were acquired on an
20 ABI 373A automated DNA sequencer (ABI) and processed using the PRISM sequence analysis software (ABI). The nucleotide sequences disclosed herein represent the range of highest quality data acquired in one pass for each clone. All DNA sequence data are reported with the same directionality, 5'
25 to 3', regardless of which strand (i.e., coding or anti-coding) is sequenced. Some DNA sequence is reported using standard IUB codes in cases where sequence ambiguities could not be absolutely resolved in first-pass sequence.

For the sequences identified herein as SEQ ID NO. 1-105, the sequences corresponding to each complementing clone identify and provide the coding sequence (gene) responsible for providing that complementation. Therefore, the sequences corresponding to each complementing clone correspond to a particular essential gene.

G. DNA sequence analysis of complementing clones

Similarity searching (general)

Sequence data were analyzed for similarity to existing publicly-available database entries both at the nucleic acid level and the (putative) polypeptide level; the current releases and daily cumulative updates of these databases are maintained at the NCBI and are freely accessible. The programs BLASTN (Altschul, et al., 1990, *J. Mol. Biol.* 215:403-410) and FASTA (Pearson, et al., 1988, *Proc. natl. Acad. Sci. USA* 85:2444-2448) were used to search the nucleic acid databases GenBank (Release 89.0) and EMBL (Rel. 43.0), while the programs BLASTX and TFASTA were used to search the protein databases SwissProt (Rel. 30.0), PIR (Rel. 45.0) and GenPept (Rel 89.0). For reporting the results of the similarity searching below, the following abbreviations of bacterial species names are used:

Bsu = *Bacillus subtilis*
Eco = *Escherichia coli*
Zmo = *Zymomonas mobilis*
Bme = *Bacillus megaterium*
Lme = *Leuconostoc mesenteroides*
Sxy = *Staph. xylosys*
Sca = *Staph. carnosus*
Sau = *Staph. aureus*
Hin = *Haemophilus influenzae*
Seq = *Strep. equisimilis*

Bca = *Bacillus caldolyticus*
Kpn = *Klebsiella pneumoniae*
Mle = *Mycobacterium leprae*

5

H. DNA Sequence of Complementing Clones

Mutant NT 6 - Clone pMP33: an example of complementing ORFs with literature precedent in *Staph. aureus*.

10 The ORF complementing the heat-sensitive phenotype of *S. aureus* mutant NT6 described here was identified by sequencing subclones of pMP33, an *E. coli*/*S. aureus* shuttle vector containing a 2.3 kilobase-pair (kb) insert of parental (i.e. wild-type) genomic DNA. The
15 subclones, pMP1006 (0.5kb), pMP1007 (0.9 kb) and pMP 1008 (0.9 kb), were generated by EcoRI and HindIII digestion of the parent clone and ligation into pGEM3Zf(+), a commercially available vector (Promega, Inc.) suitable for double-stranded DNA sequencing applications.

20 PCR-based methods (PRISM Dye Primer DNA Sequencing Kit; ABI, Inc.) were employed to generate DNA sequence data from the SP6 promoter of each of the subclones. Electrophoresis and detection of fluorescently-labelled DNA sequence ladder on an ABI 373A automated DNA
25 sequencer (ABI, Inc.) yielded the following sequence data:

SEQ ID NO. 4

subclone 1006, a 500 kb Hind III fragment

1006.seq Length: 400 nt

1 AAATAATCTA AAAATTGGTA GTNCTCCTTC AGATAAAAAT CTTACTTTAA

51 CACCATTCTT TTNAACTNNT TCCGTGTTTC TTTTCTAAG TCCATCCATA
 101 TTTTNAATGA TGTCATCTGC TGTTTTATCT TTTAAATCTA AACTGAGTG
 151 ATAACGGATT TGTCACACAG GATCAAATCC TTTATGGAAT CCAGTATGTT
 201 CAAATCCTAA GTTACTCATT TTATCAAAGA ACCAATCATT ACCAGCATT
 5 251 CCTGTAATCT CGCCATCATG ATTCAAGTAT TGATATGGTA AATATGGATC
 301 GNTATGTAGG TATAGNCAAC GATGTTTTTT AACATATTTT GGATAATTCA
 351 TTAAAGNAAA AGTGACGAG TNCTTGATTT TCATANTCAA TCACTGGACC

SEQ ID NO. 5

10 subclone 1007, a 900 bp Hind III fragment

1007.seq Length: 398 nt

1 TGCCTGAAAT NACTGTATGG CNTGCNATCT GTAAAGGCAC CAACTCTTT
 51 AGCTGTAAA TTTGTAACT TCATTATCAT TACTCCTATT TGTCTCTCGT
 101 TAATTAATTT CATTTCCGTA TTTGCAGTTT TCCTATTTCC CCTCTGCAA
 15 151 TGTCAAAAAT AATAAATCTA ATCTAAATAA GTATACAATA GTTAATGTTA
 201 AAATAAAAC ATAAACGCTT TAATTGCGTA TACTTTTATA GTAATATTTA
 251 GATTTTNGAN TACAATTTCA AAAAAAGTAA TATGANGCCTT TGGGTTTGCN
 301 CATATTACTT TTTTNGAAAT TGTATTCAAT NTTATAATTC ACCGTTTTTC
 351 ACTTTTNC A ACAGTATTC GCCTANTTTT TTAAATCAA GTAAACTT

20

SEQ ID NO. 6

subclone 1008, a 920 bp EcoR I/ Hind III fragment

1008.seq Length: 410 nt

1 GTAATGACAA ATNTAACTAC AATCGCTTAA AATATTACAA AGACCGTGTG
 25 51 TNAGTACCTT TAGCGTATAT CAACTTTAAT GAATATATTA AAGAACTAAA
 101 CGAAGAGCGT GATATTTTAA ATAAAGATTT AAATAAAGCG TTAAAGGATA
 151 TTGAAAAACG TCCTGAAAAT AAAAAAGCAC ATAACAAGCG AGATAACTTA
 201 CAACAACAAC TTGATGCAAA TGAGCAAAAG ATTGAAGAAG GTAAACGTCT
 251 ACAAGANGAA CATGGTAATG AATTACCTAT CTCTNCTGGT TTCTNCTTTA
 30 301 TCAATCCATT TGANGTTGTT TATTATGCTG GTGGTACATC AAATGCATTC
 351 CGTCATTTN CCGGAAGTTA TGCAGTGCAA TGGGAAATGA TTAATTATGC
 401 ATTAAATCAT

35

A partial restriction map of clone pMP33 appears in Fig.
 23, with open boxes to represent the percentage of the
 clone for which DNA sequence has been obtained in one pass.

40

Analysis of these data reveals identity (> 90%,
 including sequence ambiguities in first-pass sequence) at
 both the nucleotide and (predicted) amino acid-level to the
 femA gene of *S. aureus* (Genbank ID M23918; published in
 Berger-Baechi, B. et al., Mol. Gen. Genet. 219 (1989) 263-

269). The nucleotide sequence identities to the Genbank entry indicate that complementing clone pMP33 contains the complete ORF encoding the FemA protein along with the necessary upstream elements for its expression in *S.*

- 5 aureus. The figure demonstrates the relative positions of the subclones along with the location of the ORF encoding the FemA protein.

10 Mutant NT64/Clone pMP98: an example of complementing ORFs without direct literature precedent, but identifiable by similarity to genes from other bacteria

- The ORF(s) complementing the heat-sensitive phenotype of *S. aureus* mutant NT64 described here were identified by sequencing a subclone of pMP98, an *E. coli*/*S.*
- 15 aureus shuttle vector containing a 2.9 kb insert of parental (i.e. wild-type) genomic DNA. The subclone, pMP1038, was generated by EcoRI and HindIII digestion of pMP98 and ligation into pGEM3Zf(+), a commercially available vector (Promega, Inc.) suitable for use in
- 20 automated fluorescent sequencing applications. Using fluorescently-labelled dye primers (T7 and SP6; ABI, Inc.), a total of 914 bp of sequence from the two edges of the subclone was generated.

25 SEQ ID NO. 106
subclone 1038, a 2800 bp genomic fragment
1038.sp6 Length: 417 nt

30 1 GTGATGGATT AAGTCCTAAA TTNNATTTCG CTTTCTTGTC TTTTAAATCT
51 TTTTCAGACA TTTTATCGAT TTCACGTTTT GTATACTTAG GATTTAAATA
101 GGCATTAATT GTTTTCTTGT CCAAAAATTG ACCATCTTGA TACAAATATT
151 TATCTGTGG AAATACTTCT TTAATAAGT NCAATAAACC ATCTTCAAAG

201 TCGCCGCCAT TATAACTATT TGCCATGTGA TCTTGTA AAA GTCCTCTTGC
 251 CTGGNTTCT TTAATGGTA ACAATGTACG NTAGTTATCA CCTTGACAT
 301 TTTTATCCGT TGCAATTTCT TNTACTTGAT TTGAACTATT GTTATGTTTT
 351 NAATTATCTT TTCCCAGCCT GGGTCATCCT TATGGTTANC ACAAGCAGCG
 5 401 AGTATAAAGG TAGCTGT

SEQ ID NO. 107

1038.t7 Length: 497 nt

1 TAATGTAGCA ATTACAAGGC CTGAAGAGGT GTTATATATC ACTCATGCGA
 10 51 CATCAAGAAT GTNATTTGGN CGCCCTCAGT CAAATATGCC ATCCAGNTTT
 101 TNAAAGGAAA TTCCAGAATC ACTATTAGAA AATCATTCAA GTGGCAAACG
 151 ACAACGGTA CAACCTNNGG CAAAACCTTT TNCTAAACGC GGNTTTTGTC
 201 AACGGNCAAC GTCAACGGNN AANCAAGTAT TTNATCTGN TTGGAATNTT
 251 GGTGGCAANG TGGTGCNTAA NGNCNCCGGG GGGAGGCATT GTNNGTAATT
 15 301 TTAACGNGGA NAATGGCTCN NTCGGNCTNG GTNTTATNTT TTATTCACAC
 351 AGGGNCGCGN CANGTTTTTT TTGTNGGATT TTTTCCCCC NTTTTTNA
 401 AGNGGGGTN TTNNGGGTGG CTGNTTTANT NGTCTCNGNG TGGNCGTGN
 451 TCATTNNTTT TTTTNTTNA TCCAAGCCTT NTATGACTTT NNTTGGG

20 Similarity searches at the nucleotide and
 (putative) amino acid level reveal sequence identity from
 the left-most (T7) edge of the clone to the Genbank entry
 for *pcrA*, a putative helicase from *S. aureus* (Genbank ID
 M63176; published in Iordanescu, S.M. and Bargonetti, J. J.
 25 *Bacteriol.* 171 (1989) 4501-4503). The sequence identity
 reveals that the pMP98 clone contains a C-terminal portion
 of the ORF encoding *pcrA*, but that this ORF is unlikely to
 be responsible for complementation of the NT64 mutant.
 The Genbank entry extends 410 bp beyond the 3' end of the
 30 *pcrA* gene, and does not predict any further ORFs.
 Similarity searches with data obtained from the right-most
 (SP6) edge reveal no significant similarities, indicating
 that the complementing ORF in pMP98 is likely to be
 unpublished for *S. aureus*. A partial restriction map of
 35 clone pMP98 appears in Fig. 20 (there are no apparent
 restriction sites for BamH I, EcoR I, or Hind III); the

relative position and orientation of the identified (partial) ORF corresponding to the PcrA protein is indicated by an arrow:

From the preliminary sequence data, the following
5 PCR primers were designed:

pMP98(+): 5' - CTG AAG AGG TGT TAT ATA TCA C - 3'
pMP98(-): 5' - GTG ATG GAT TAA GTC CTA AAT T - 3'

These primers were used to amplify the 2.9 kb
10 genomic DNA fragment in one round of PCR amplification directly from *S. aureus* genomic DNA (parental strain 8325-4). Similar strategies using PCR primers designed from partial sequences can be used for amplifying the genomic sequence (or a cloned genomic sequence) corresponding to
15 the additional complementing clones described below. Additional primers based upon the obtained sequence were designed to generate further DNA sequence data by primer-walking, using the dye terminator strategy (PRISM DyeDeoxy Terminator Kit; ABI, Inc.).

20 pMP98.b(+): 5' - CTC AGT CAA ATA TGC CAT CCA G - 3'
pMP98.b(-): 5' - CTT TAA ATG GTA ACA ATG TAC G - 3'

The following sequence data were obtained, as depicted in the partial restriction map in Fig. 41:

25

clone pMP98
SEQ ID NO. 36

30

pMP98 Length: 2934 nt

1 CATGAAATGC AAGAAGAACG TCGTATTTGT TATGTAGCAA TTACAAGGGC

51 TGAAGAGGTG TTATATATCA CTCATGCGAC ATCAAGAATG TTATTTGGTC
 101 GCCCTCAGTC AAATATGCCA TCCAGATTTT TAAAGGAAAT TCCAGAATCA
 151 CTATTAGAAA ATCATTCAAG TGGCAAACGA CAAACGATAC AACCTAAGGC
 201 AAAACCTTTT GCTAAACGCG GATTTAGTCA ACGAACAACG TCAACGAAAA
 5 251 AACAAGTATT GTCATCTGAT TGGAAATGTAG GTGACAAAGT GATGCATAAA
 301 GCCTGGGGAG AAGGCATGGT GAGTAATGTA AACGAGAAAA ATGGCTCAAT
 351 CGAACTAGAT ATTATCTTTA AATCACAAGG GCCAAAACGT TTGTTAGCGC
 401 AATTTGCACC AATTGAAAAA AAGGAGGATT AAGGGATGGC TGATTTATCG
 451 TCTCGTGTGA ACGRDTTACA TGATTTATTA AATCAATACA GTTATGAATA
 10 501 CTATGTAGAG GATAATCCAT CTGTACCAGA TAGTGAATAT GACAAATTAC
 551 TTCATGAACT GATTAAAATA GAAGAGGAGC ATCCTGAGTA TAAGACTGTA
 601 GATTCTCCAA CAGTTAGAGT TGGCGGTGAA GCCCAAGCCT CTTTCAATAA
 651 AGTCAACCAT GACACGCCAA TGTTAAGTTT AGGGAATGCA TTTAATGAGG
 701 ATGATTTGAG AAAATTTCGAC CAACGCATAC GTGAACAAAT TGGCAACGTT
 15 751 GAATATATGT GCGAATTAAA AATTGATGGC TTAGCAGTAT CATTGAAATA
 801 TGTTGATGGA TACTTCGTTT AAGGTTTAAAC ACGTGGTGAT GGAACAACAG
 851 GTTGAAGATA TTACCGRAAA TTTAAAAACA ATTTCATGCGA TACCTTTGAA
 901 AATGAAAGAA CCATTAAATG TAGAAKTYCG TGGTGAAGCA TATATGCCGA
 951 GACGTTCAAT TTTACGATTA AATGAAGAAA AAGAAAAAAA TGATGAGCAG
 20 1001 TTATTTGCAA ATCCAAGAAA CGCTGCTGCG GGATCATTAA GACAGTTAGA
 1051 TTCTAAATTA ACGGCAAAAC GAAAGCTAAG CGTATTTATA TATAGTGTCA
 1101 ATGATTTTAC TGATTTCAAT GCGCGTTCGC AAAGTGAAGC ATTAGATGAG
 1151 TTAGATAAAT TAGGTTTTAC AACGAATAAA AATAGAGCGC GTGTAAATAA
 1201 TATCGATGGT GTTTTAGAGT ATATTGAAAA ATGGACAAGC CAAAGAAGAG
 25 1251 TTCATTACCT TATGATATTG ATGGGATTGT TATTAAGGTT AATGATTTAG
 1301 ATCAACAGGA TGAGATGGGA TTCACACAAA AATCTCCTAG ATGGGCCATT
 1351 GCTTATAAAT TTCCAGCTGA GGAAGTAGTA ACTAAATTAT TAGATATTGA
 1401 ATTAAGTATT GGACGAACAG GTGTAGTCAC ACCTACTGCT ATTTTAGAAC
 1451 CAGTAAAAGT AGCTGGTACA ACTGTATCAA GAGCATCTTT GCACAATGAG
 30 1501 GATTTAATTC ATGACAGAGA TATTCGAATT GGTGATAGTG TTGTAGTGAA
 1551 AAAAGCAGGT GACATCATAC CTGAAGTTGT ACGTAGTATT CCAGAACGTA
 1601 GACCTGAGGA TGCTGTCACA TATCATATGC CAACCCATTG TCCAAGTTGT
 1651 GGACATGAAT TAGTACGTAT TGAAGGCGAA GTTAGCACTT CGTTGCATTA
 1701 ATCCAAAATG CCAAGCACAA CTTGTTGAAG GATTGATTCA CTTTGTATCA
 35 1751 AGACAAGCCA TGAATATTGA TGGTTTAGGC ACTAAAATTA TTCAACAGCT
 1801 TTATCAAAGC GAATTAATTA AAGATGTTGC TGATATTTTC TATTTAACAG
 1851 AAGAAGATTT ATTACCTTTA GACAGAATGG GGCAGAAAAA AGTTGATAAT
 1901 TTATTAGCTG CCATTCAACA AGCTAAGGAC AACTCTTTAG AAAATTTATT
 1951 ATTTGGTCTA GGTATTAGGC ATTTAGGTGT TAAAGCGAGC CAAGTGTGKAG
 40 2001 CAGAAAAATA TGAAACGATA GATCGATTAC TAACGGTAAC TGAAGCGGAA
 2051 TTAGTAGAAT TCATGATATA GGTGATAAAG TAGCGCAATC TGTAGTTACT
 2101 TATTTAGCAA ATGAAGATAT TCGTGCTTTA ATTCCATAGG ATTTAAAGAT
 2151 AAACATGTTA ATATGATTTA TGAAGGTATC CAAAACATCA GATATTGAAG
 2201 GACATCCTGA ATTTAGTGGT AAAACGATAG TACTGACTGG TAAGCTACAT
 45 2251 CCAAATGACA CGCAATGAAG CATCTAAATG GCTTGCATCA CCAAGGTGCT
 2301 AAAGTTACAA GTAGCGTTAC TAAAAATACA GATGTCGTTA TTGCTGGTGA
 2351 AGATGCAGGT TCAAAATTAA CAAAAGCACA AAGTTTAGGT ATTGAAATTT
 2401 GGACAGAGCA ACAATTTGTA GATAAGCAAA ATGAATTAAA TAGTTAGAGG
 2451 GGTATGTCGA TGAAGCGTAC ATTAGTATTA TTGATTACAG CTATCTTTAT
 50 2501 ACTCGCTGCT TGTGGTAACC ATAAGGATGA CCAGGCTGGA AAAGATAATC
 2551 AAAAACATAA CAATAGTTCA AATCAAGTAA AAGAAATTGC AACGGATAAA

2601 AATGTACAAG GTGATAACTA TCGTACATTG TTACCATTTA AAGAAAGCCA
 2651 GGCAAGAGGA CTTTTACAAG ATAACATGGC AAATAGTTAT AATGGCGGCG
 2701 ACTTTGAAGA TGGTTTATTG AACTTAAGTA AAGAAGTATT TCCAACAGAT
 2751 AAATATTTGT ATCAAGATGG TCAATTTTGG GACAAGAAAA CAATTAATGC
 5 2801 CTATTTAAAT CCTAAGTATA CAAAACGTGA AATCGATAAA ATGTCTGAAA
 2851 AAGATAAAAA AGACAAGAAA GCGAATGAAA ATTTAGGACT TAATCCATCA
 2901 CACGAAGGTG AAACAGATCG ACCTGCAGKC ATGC

From this data, a new ORF in the pMP98 clone was
 10 identified as having significant similarity to *lig*, the
 gene encoding DNA ligase from *E. coli*: (Genbank ID M30255;
 published in Ishino, Y., et al., *Mol. Gen. Genet.* 204 (1986), 1-
 7). The revised clone map of pMP98, including the predicted
 size and orientation corresponding to the putative DNA
 15 ligase ORF, is shown in Fig. 41:

The DNA ligase protein from *E. coli* is composed
 of 671 amino acids; a polypeptide translated from *S. aureus*
 DNA sequence acquired above matches the C-terminal 82 amino
 acids of the *E. coli* DNA ligase with a 52% sequence
 20 identity and a 67% sequence similarity; this level of
 similarity is considered significant when comparing
 proteins from Gram-negative and Gram-positive bacteria.
 Since the predicted coding region of the *S. aureus* gene for
 DNA ligase is small enough to be contained within clone
 25 pMP98 and the gene for DNA ligase is known to be essential
 to survival for many bacterial species, NT64 is concluded
 to contain a *ts* mutation in the gene for DNA ligase.

Mutant NT42/Clone pMP76: an example of
complementing ORFs with unknown function

The ORF(s) complementing the temperature-sensitive phenotype of *S. aureus* mutant NT42 described here was identified by sequencing subclones of pMP0076, an *E. coli*/*S. aureus* shuttle vector containing a 2.5 kb insert of parental (i.e. wild-type) genomic DNA. The subclones, pMP1026 (1.1 kb) and pMP1027 (1.3 kb), were generated by EcoRI and BamHI digestion of the parent clone and ligation into pGEM3Zf(+), a commercially available vector (Promega, Inc.) suitable for double-stranded DNA sequencing applications.

PCR-based methods (PRISM Dye Primer DNA Sequencing Kit; ABI, Inc.) were employed to generate DNA sequence data from the SP6 and T7 promoters of both of the subclones. Primer walking strategies were used to complete the sequence contig. Electrophoresis and detection of fluorescently-labelled DNA sequence ladder on an ABI 373A automated DNA sequencer (ABI, Inc.) yielded the following sequence data:

clone pMP76
SEQ ID NO. 37

pMP76 Length: 2515 nt

```

1  CSYCGGWACC CGGGGATCCT CTAGAGTCGA TCGTTCAGG ACGTATTCGA
51  ACTTATAATT ATCCACAAAG CCGTGTAACA GACCATCGTA TAGGTCTAAC
101 GCTTCAAAAA TTAGGGCAAA TTATGGAAGG CCATTTAGAA GAAATTATAG
151 ATGCACTGAC TTTATCAGAG CAGACAGATA AATTGAAAGA ACTTAATAAT
201 GGTGAATTAT AAAGAAAAGT TAGATGAAGC AATTCATTTA ACACAACAAA
251 AAGGGTTTGA ACAAACACGA GCTGAATGGT TAATGTTAGA TGTATTTCAA
301 TGGACGCGTA CGGACTTTGT AGTCCACATG CATGATGATA TGCCGAAAGC

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351 GATGATTATG AAGTTCGACT TAGCATTACA ACGTATGTTA TTAGGGAGAG
 401 CCTATACAGT ATATAGTTGG CTTTGCCTCA TTTTATGGTA GAACGTTTGA
 451 TGTAAACTCA AATTGTTTGA TACCAAGACC TGAAACTGAA GAAGTAATGT
 501 TGCATTTCTT ACAACAGTTA GAAGATGATG CAACAATCGT AGATATCGGA
 5 551 ACGGGTAGTG GTGTACTTGC AATTACTTTG AAATGTTGAA AAGCCGGATT
 601 TAAATGTTAT TGCTACTGAT ATTTCACTTG AAGCAATGAA TATGGCTCCG
 651 TAATAATGCT GAGAAGCATC AATCACAAAT ACAATTTTTA ACAGGGGATG
 701 CATTAAAGCC CTTAATTAAT GAAGGTATCA AKTTGAACGG CTTTGATATC
 751 TAATCCMCCA TATATAGATG AAAAAGATAT GGTACGATG TCTCCMACGG
 10 801 TTACGARATT CGAACCACAT CAGGCATTGT TTGCAGATAA CCATGGATAT
 851 GCTATTTATG AATCAATCAT GGAAGATTTA CCTCACGTTA TGGAAAAAGG
 901 CAGCCCAGTT GTTTTTGAAA TTGGTTACAA TCAAGGTGAG GCACTTAAAT
 951 CAATAATTTT AAATAAATTT CCTGACAAAA AAATCGACAT TATTAAAGAT
 1001 ATAAATGGCC ACGATCGAAT CGTCTCATTT AAATGGTAAT TAGAAGTTAT
 15 1051 GCCTTTGCTA TGATTAGTTA AGTGCATAGC TTTTGCCTTT ATATTATGAT
 1101 AAATAAGAAA GCGGTGATTA AGTTGGATAC TAAAATTTGG GATGTTAGAG
 1151 AATATAATGA AGATTTACAG CAATATCCTA AAATTAATGA AATAAAAGAC
 1201 ATTGTTTTAA ACGGTGGTTT AATAGGTTTA CCAACTGAAA CAGTTTATGG
 1251 ACTTGCAGCA AATGCGACAG ATGAAGAAGC TGTAGCTAAA ATATATGAAG
 20 1301 CTAAAGGCCG TCCATCTGAC AATCCGCTTA TTGTTTCATAT ACACAGTAAA
 1351 GGTCAATTAA AAGATTTTAC ATATACTTTG GATCCACGCG TAGAAAAGTT
 1401 AATGCAGGCA TTCTGGCCGG GCCCTATTTT GTTTTATATTG CCGTTAAAGC
 1451 TAGGCTATCT ATGTCGAAAA GTTCTGGAG GTTTATCATC AGTTGCTGTT
 1501 AGAATGCCAA GCCATTCTGT AGGTAGACAA TTATTACAAA TCATAATGA
 25 1551 ACCTCTAGCT GCTCCAAGTG CTAATTTAAG TGGTAGACCT TCACCAACAA
 1601 CTTTCAATCA TGTATATCAA GATTTGAATG GCCGTATCGA TGGTATTGTT
 1651 CAAGCTGAAC AAAGTGAAGA AGGATTAGAA AGTACGGTTT TAGATTGCAC
 1701 ATCTTTTCCT TATAAAATTG CAAGACCTGG TTCTATAACA GCAGCAATGA
 1751 TTACAGAAAT AMTTCCGAAT AGTATCGCCC ATGCTGATTA TAATGATACT
 30 1801 GAACAGCCAA TTGCACCAGG TATGAAGTAT AAGCATTACT CAACCCAATA
 1851 CACCACTTAC AATTATTACA GATATTGAGA GCAAAATTGG AAATGACGGT
 1901 AAAGATTRKW MTTCTATAGC TTTTATTGTG CCGAGTAATA AGGTGGCGTT
 1951 TATACCAAGT GARSCGAAT TCATTCAATT ATGTCAGGAT GMCAATGATG
 2001 TTAAACAAGC AAGTCATAAT CTTTATGATG TGTTACATTC ACTTGATGAA
 35 2051 AATGAAAATA TTTCAGCGGC GTATATATAC GGCTTTGAGC TGAATGATAA
 2101 TACAGAAGCA ATTATGAATC GCATGTTAAA AGCTGCAGGT AATCACATTA
 2151 TTAAAGGATG TGAACATGA AGATTTTATT CGTTTGTACA GGTAACACAT
 2201 GTCGTAGCCC ATTAGCGGGA AGTATTGCAA AAGAGGTTAT GCCAAATCAT
 2251 CAATTTGAAT CAAGAGGTAT ATTCGCTGTG AACAATCAAG GTGTTTCGAA
 40 2301 TTATGTTGAA GACTTAGTTG AAGAACATCA TTTAGCTGAA ACGACCTTAT
 2351 CGCAACAATT TACTGAAGCA GATTTGAAAG CAGATATTAT TTTGACGATG
 2401 TCGTATTCGC ACAAAGAATT AATAGAGGCA CACTTTGGTT TGCAAAATCA
 2451 TGTTTTCACA TTGCATGAAT ATGTAAAAGA AGCAGGAGAA GTTATAGATC
 2501 GACCTGCAGG CATGC

45

Analysis of the DNA sequence data at the
 nucleotide level reveals no significant similarity to data
 in the current release of the Genbank or EMBL databases.

Analysis of the predicted ORFs contained within clone pMP76 reveals a high degree of similarity to two open reading frames identified in *B. subtilis*; "ipc29D" and "ipc31D" (EMBL entry Z38002). A partial restriction map of pMP76 is depicted in Fig. 42, along with an open box to indicate the percentage of the clone for which DNA sequence has been obtained. The relative orientation and predicted size of the "ipc29D" ORF is indicated by an arrow:

These two ORFs identified from the EMBL entry Z38002 were predicted from genomic sequence data and are denoted as "putative"; no characterization of expression or function of the predicted gene products has been reported in the literature. A similarity has been noted between the predicted Ipc31D-like polypeptide and the SUA5 gene product from yeast (*S. cerevisiae*), but functional characterization still remains to be performed. Hence, the ORFs contained within clone pMP76 represent putative polypeptides of uncertain function, but are known to be responsible for restoring a wild-type phenotype to NT42.

In addition to the illustrative sequences described above, the following sequences of clones complementing heat sensitive mutants of *S. aureus* similarly provide essential genes.

Mutant: NT3

Phenotype: temperature sensitivity

Sequence map: Mutant NT3 is complemented by plasmid pMP27, which contains a 3.9 kb insert of *S. aureus* genomic DNA. The partial restriction map of the insert is depicted in

Fig. 21; open boxes along part of the length of the clone indicate the portions of the clone for which DNA sequence has been obtained (this contig is currently being completed). Database searches at both the nucleic acid and protein levels reveal strong similarity at both the peptide and nucleic acid level to the C-terminal fragment of the SecA protein from *S. carnosus* (EMBL Accession No. X79725) and from *B. subtilis* (Genbank Accession No. D10279). Since the complete SecA ORF is not contained within clone pMP27, SecA is unlikely to be the protein responsible for restoring mutant NT3 to a wild-type phenotype. Further strong peptide-level similarities exist between the DNA sequence of a Taq I subclone of pMP27 and the *prfB* gene, encoding Peptide Release Factor II, of *B. subtilis* (Genbank D10279; published in Pel et al., 1992, *Nucl. Acids Res.* 20:4423-4428). Cross complementation analysis (data not shown) suggests that a mutation in the *prfB* gene is most likely to be responsible for conferring a temperature-sensitive phenotype to mutant NT3 (i.e. it is an essential gene).

DNA sequence data: The following DNA sequence data represents the sequences at the left-most and right-most edges of clone pMP27, using standard M13 forward and M13 reverse sequencing primers, and then extending via primer walking strategies. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP27 (forward and reverse contigs)

SEQ ID NO: 1

pMP27.forward Length: 1739 nt

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1   CTCGCAGCCG NYAKYCGWAA ATGGTCCAAT GTACTCCATC CATCACTGCA
35  51   TCAACCTTAC CTGTTTCTTC GTTCGTACGA TGATCTTTCA CCATTGAGTA
    101  TGGATGGAAA ACATATGATC TAATTTGGCT TCCCCAGCCG ATTTCTTTTT
    151  GTTCGCCACG AATTTTCAGCC ATTTACGTG CCTGCTCTTC CAATTTTAAT
    201  TGATATAATT TAGACTTTAA CATTTTCATA GCTGCTTCAC GGGTTTTAAT
    251  TTGAGAACGT TCATTTTGGT TATTAACAAC TATACCTGAG GGGTGGTGGG
40  301  TAATTCGTAT TGCCGATTCA GTTTTGTTAA TATGCTGACC ACCTGCACCA
    351  GAAGCTCTGA ATGTATCAAC TGTAATATCA TCCGGATTGA TTTCAATCTC
    401  TATTTTCATCA TTATTTAAAT CTGGAATAAC GTCGCATGAT GCAAATGATG
    451  TATGACGACG TCCTGATGAA TCAAATGGAG AAATTCGTAC TAGTCGGTGT
    501  ACACCTTTTT CAGCTTTTAA ATAACCATAA GCATTATGCC CTTTGATGAG
45  551  CAATGTTACA CTTTTAATCC CCGCTTCATC CCCAGGTAGA TAATCAACAG

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601 TTTCAACTTT AAAGCCTTTC TTCTCAACAA TAACGTTGAT ACATTCTAAA
 651 TAGCATATTA GCCCAATCTT GAGACTCCGT GCCACCTGCA CCAGGATGTA
 701 ACTCTAGAAT TGCGTTATTG GCATCGTGAG GCCCATCTAA TAATAATTGC
 751 AATTCGTATT CATCCACTTT AGCCTTAAAA TTAATGACCT CTTGCTCTAA
 801 GTCTTCTTTC ATTTCCCTCA TCAAATTCCT CTTGTAATAA ATCCCAAGTA
 851 GCATCCATGT CATCTACTTC TGCTTGAGT GTTTTATAAC CATTAECTAT
 901 TGCTTTTAAAC GCATTATTTT TATCTATAAT ATCTTGCGCT TTCGTTTGGT
 951 TATCCCAAAA ATTAGGTTCT GCCATCATT CTTCATATTC TTGAATATTA
 1001 GTTTCTTTGT TCTCTAAGTC AAAGAGACCC CCTAATTTGT GTTAAATCTT
 1051 GATTATACTT ATCTATATTT CGTTTGATTT CTGATAATTC CATAGCATTC
 1101 GCTCCTATTT ATATTTCAAT TCAAGTCATT GATTTGCATC TTTTATAATG
 1151 CTAAATTTTA ACATAATTTT GTTAAATAAC AATGTTAAGA AATATAAGCA
 1201 CACTGACAAT TAGTTTATGC ATTTATTGTT TAAAAAWGCA GTACATTTAT
 1251 GCATCGACAT ATGCCATAAC CGATTTTTTA AAACTAAGTA CATAACAACG
 1301 TTAAACAAC TCTTCACATT TTTTAAAGTA TTAAACGCTT GTAAAAATAA
 1351 AAGACTCCTC CCATAACACA AACTATAGGT GTTTAATTGG AAGGAGTTAT
 1401 TTTATATCAT TTATTTTCCA TGGCAATTTT TGAATTTTTT ACCACTACCA
 1451 CATGGACAAT CATCGTTACG ACCAACTTGA TCGCCTTTAA CGATTGGTTT
 1501 CGGTTTCACT TTTTCTTTAC CATCTTCAGC TGAAACGTGC TTCGCTTCAC
 1551 CAAACTCTGT TGTTTTTTCA CGTTCAATAT TATCTTCAAC TTGTACTACA
 1601 GATTTTAAAA TGAATTTACA AGTATCTTCT TCAATATTTT GCATCATGAT
 1651 ATCAAATAAT TCATGACCTT CATTTTGATA GTCACGTAAT GGATTTTGTT
 1701 GTGCATAAGA ACGTAAGTGA ATACCTTGAC GTAATTGAT

25 pMP27.reverse Length: 2368 nt
 SEQ ID NO. 2

1 CTGCAGGTCG ATCTGCATCT TGATGTTTAT GAAATTCGAG TTGATCTAGT
 51 AATTAAATAA CCAGCTAATA ATGACACTAC ATCAGKAAGA ATAATCCACT
 101 CGTTATGGAA ATACTCTTTA TAGATTGAGG CACCAATTAA AATTAATGTC
 151 AGAATAGTAC CGACCCATTT ACTTCTTGTT ATTACACTAA ATAATACTAC
 201 CAAGACACAT GGAAAGAATG CTGCGCTAAA ATACCATATC ATTCATTTTC
 251 CTCTTTTCTT TTATTTAAAA TGTTCAATGGT TGTTTCTCTT AATTCTGTTT
 301 TAGGTATAAA GTTTTCAGTC AACATTTCTG GAATGATATT ATTAATAAAA
 351 TCTTGACAG ATGCTAAATG GTCAAATTGA ATAATTGTTT CTAGACTCAT
 401 TTCATAAATT TCGAAAAATA ATTCTTCGGG ATTACGKTTT TGTATTTCTC
 451 CAAATGTTTC ATAAAGCAAA TCAATTTTAT CAGCAACTGA AAGTATTTGG
 501 CCTTCTAATG AATCATCTTT ACCTTCTTGC AGTCGTTGCT TATAAACATC
 551 TCTATATTGT AATGGAATTT CTTCTTCAAT AAAGGTCTCT ACCATTTCTT
 601 CTTCAACTTG CGAAAATAAT TTTTAAAT CACTACTCGC ATATTTAACA
 651 GGTGTTTTTA TATCACCAGT AAACACTTCG GSGAAATCAT GATTTAATGC
 701 TTTTTCATAT AAGCTTTTCC AATTAAYCTT TCTCCATGAT ATTCTTCAAC
 751 TGTTGCTAGA TATTGTGCAA TTTTAGTTAC TTTAAAGGAG TGTGCTGCAA
 801 CATTGTGTTT AAAATATTTA AATTTTCCAG GTAATCTTAT AAGTCTTTCC
 851 ATATCTGATA ATCTTTTAAA ATATTGATGT ACACCCATTT CAATTACCTC
 901 CTCCATTAAT TAATCATAAA TTATACTTTC TTTTACATA TCAATCAATT
 951 AAATATCATT TAAATATCTT CTTTATATAA CTCTGATTAA ATGATACCAA
 1001 AAAATCCTCT CAACCTGTTA CTTAAACAGG CTAAGAGGGT AGTCTTGTCT
 1051 TGATATATTA CTTAGTGGAT GTAATTATAT TTTCTGGAT TTAAATTTGT
 1101 TCTTGAAGAT TTAACATTAA ATCCAGCATA GTTCATTTTC AGAAACAGTA
 1151 ATTGTTCCMT TTAGGGTTTA CAGATTCAAC AACACCAACA TGTCCATATG
 1201 GACCAGCAGC TGTTTGAAAA ATAGCGCCAA CTTCTGGKGT TTTATCTACT

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1251 TTTAAATCCT GCAACTTTTG CTGCGTAATT CCAGTTATTT GCATTGCCCC
1301 ATAAACTTCC TATACTTCTA CCTAATTGTG CACGACGATC GAAAGCATAA
1351 TATGTGCAGT TTCCATAAGC ATATAAGTTT CCTCTGTTAG CAACTGATTT
1401 ATGTAGTGA TGTGCAACAG GTACAGTTGG TACTGATTTT TGTACTTGAG
5 1451 CAGGTTTGTA TGCTACATTA ACTGTCTTAG TTACTGCTTG CTTAGGTGCT
1501 TGCTTAACTA CTACTTTTTT AGATGCTTGT TGTACAGGTT GTTTTACTAC
1551 CTTTTTAGCT TGGCTTGCTT TTCTTACTGG TGATTTAACC GCTTTAGTTT
1601 GTTTCACTTT ATTTTGAGGC ACAAGTGAAA TCACGTCACC AGGAAAAATT
1651 AAAGGTGTTA CACCAGGATT GTATTGAATA TAATTGATTC AACGTAAAGT
10 1701 GATGCTCTTA AAGCAATCTT ATATTAATGA ATCGCCAGCA ACTACTGTWT
1751 AAGTTGTCGG TGATTGCGTT TGTGCTTGAA CATTGATAC ATAATTATGT
1801 TGAACAGGTG TTTTACTTGG TGTGCCATGT TGTTGTGCAT GTGCKGCATT
1851 ATTTAAAGCK AAAAAAGCTA ACACTGACGA AACCGTCACT GWAAGARART
1901 TTTTCATCTK GCTGTCATTC CTTTGCTGTW AGTATTTTAA GTTATGCAAA
15 1951 TACTATAGCA CAATACATTT TGTCCAAAAG CTAATTGTGA TAACGANGTA
2001 ATCAAATGGT TAACAANATN AANAGAAGAC AACCGTNTAT CATAGNGGNA
2051 AANGTAGNCA TACCATGNAA TTGAGAACGT TNTCAANAAN TAANTCAATA
2101 CCNTGAAAAT CGCCATAGGN AATATTACNA AATGCACACT GCATATGNTG
2151 NTTTAACAAA CACNACTTTT NANAAATATA NTCTAACTCT ATCTACCGAA
20 2201 TTGNACTTAA ATATTCATAA ANAAATNATA TTCNAAAATC TAATTTACAA
2251 TTTATTTAGC TACCTTTAAA AAANCNNAAA ACCGACGNCC TTTTAGAGCC
2301 TCGGTTTTTA NATATATNTT AATCGTGCGA CATTGTCTGT TTTNAATNTG
2351 ATTCGACTCT AGNGGATC

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25

Mutant: NT5**Phenotype:** temperature sensitivity**Sequence map:** Mutant NT5 is complemented by plasmid

30 pMP628, which contains a 2.5 kb insert of *S. aureus* genomic DNA. The partial restriction map of the insert is depicted in Fig. 22. Database searches at both the nucleic acid and protein levels reveal strong similarity between one of the ORFs contained within clone pMP628 and the *zwf* gene from a

35 variety of species, which encodes the Glucose-6-Phosphate Dehydrogenase (G6PD) protein (EC 1.1.1.49). The strongest similarity is demonstrated in the Genbank entry for G6PD (Accession No. M64446; published in Lee, W.T. et al. *J. Biol. Chem.* 266 (1991) 13028-13034.) from *Leuconostoc*

40 *mesenteriodes*, here abbreviated as "Lme".

DNA sequence data: The following DNA sequence data represents the complete first-pass sequence of pMP628; the sequence below can be used to design PCR primers for the

45 purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP628

SEQ ID NO. 3

pMP628 Length: 2494 nt

5
1 AATCATTTTA AATGATTGAT CAAGATGGTA TGGCGAAAGA CCAACGTAAT
51 CACTTAATTC TTGCAAATTG AAAGGCTCTA ATAAACGATC TTCAATATAA
101 ACAATTGCCT GTTGTATTTG CTTGATAACG TCCAAAACCTT TCACTCCAAT
151 TAATTCAATC ATTTATTTTT ATTCTACATT ATTTCTATAA ATTATACACC
10 201 CATTTGTTCA ATGATTATTA AAATAGTTTT GGGCATTGTA AAATATAATT
251 TCATAATATA GTCTAGAAAA AAAGCGAATG ATAGAACAAT TGATTACTTT
301 GATTGTAAT CAATCCTTGT CATTCGCTCA TTTATTTTTG TTTAACATGT
351 GCGTTTAAAT TCAATTATTG AATATCGTCC CACCAATGGT TACCATCAGG
401 AGCAAGTAGT AAATCACTTT CTAATGGACC ATTAGTACCT GATTATAGT
15 451 TAGGGAATTC TGGATCAACC ATATTCCATT CATCTTGGAA TTGCATCAAC
501 AAATTTCCAT GTTGATTTTA ATTCTTCCCA GTGCGTGAAG TTAGTGGCAT
551 CACCTTTAAG ACAATCAAAT AATAGATTTT CATATGCATC TACAGTATTC
601 ATTTTATCTT GAGCGCTCAT TGAGTAAGAC AATTGGACAG GTTCTGTTTC
651 GATACCTTGT GTWTTTTTCT TAGCATTAR ATGTAAAGAT ACACCTTCAT
20 701 TAGGTTGGAT ATTGATTANT AATAGGTTTG AATCTAACAG TTTATCAGTT
751 TCATAGTATA AGTTCATTGG TACTTCTTTA AATTCAACGA CAACTTGAAT
801 TGTTTTAGAT TTCATACGTT TACCAGTACG GATATAGAAT GGTACACCAG
851 CCCATCTAAA GTTATCAATT GTTAATTTAC CTGAAACAAA GGTAGGTGTG
901 TTAGAGTCAT CTGCAACGCG ATCTTCATCA CGGTATGCTT TAACCTGTTT
25 951 ACCATCGATA TAGCCTTCGC CATATTGACC ACGAACAAAG TTCTTTTTAA
1001 CATCTTCAGA TTGGAAATGA CGCAGTGATT TAAGTACTTT TAACCTTCTC
1051 AGCACGGATA TCTTCACTAT TTAAACTAAT AGGTGCTTCC ATAGCTAATA
1101 ATGCAACCAT TTGTAACATG TGGTTTTGCA CCATATCTTT TAGCGCGCCA
1151 CTTGATTCAT AATAACCACC ACGATCTTCA ACACCTAGTA TTTCAGAAGA
30 1201 TGTAACYYGG ATGTTTGAAA TATATTGTT ATTCCATAAT GGTTCAAACA
1251 TCGCATTCGC AAAACGTAAT ACCTCGATAT TTTGAACCAT GTCTTTTCCT
1301 AAATAGTGGT CMATACGRTA AATTCTTCT TCTTTAAATG ATTTACGAAT
1351 TTGATTGTTT AATGCTTCGG CTGATTTTAA ATCACTACCG AATGGTTTTT
1401 CGATAACAAG GCGTTTAAAT CCTTTTGAT CAGTAAGACC AGAAGATTTT
35 1451 AGATAATCAG AAATAACGCC AAAGAATTGT GGTGCCATTG CTAAATAGAA
1501 TAGTCGATTA CCTTYTAATT CAAATTGGCT ATCTAATTCA TTACTAAAAT
1551 CTAGTAATTT CTTGATAGCT TTCTTCATTA CTAACATCAT GTCTATGATA
1601 GAAGACATGT TCCATAAACG CGTCAATTTT GTTTGTATCT TTWACGTGCT
1651 TTTGAATTGA TGATTTTAAC TTGATTACGG AAATCATCAT TAGTAATGTC
40 1701 ACGACGTCCA ATACCGATGA TGGCAATATG TTCATCTAAA TTGTCTTGTT
1751 GGTAGAGATG GAATATTGAT GGAACAACCT TACGATGGCT TAAGTCACCA
1801 GTTGCACCAA AGATTGTGAT TAAACATGGG ATGTGTTTGT TTTTAGTACT
1851 CAAGATTAAA ACCTCAATTC WYMCATTAGA TATATSATTT ATTATKAYMM
1901 GATAATCCAT TTCAGTAGGT CATACMATAT GYTGACTGT ATGCAGTKTC
45 1951 TTAAATGAAA TATCGATTCA TGTATCATGT TTAATGTGAT AATTATTAAT
2001 GATAAGTATA ACGTAATTAT CAAAATTTAT ATAGTTATGT CTAACGTTAA
2051 AGTTAGAAAA ATTAAGTAGC AAAGACGAAT TTTTAACAGA TTTTGATTCA
2101 AGTATAAATT AAAACTAAAT TGATACAAAT TTTATGATAA AATGAATTGA
2151 AGAAAAGGAG GGGCATATAT GGAAGTTACA TTTTTTGGAA CGAGTGCAGG
50 2201 TTTGCCTACA AAAGAGAGAA ATACACAAGC AATCGCCTTA AATTTAGAAC
2251 CATATTCCAA TTCCATATGG CTTTTCGACG TTGGTGAAGG TACACAGCAC

2301 CAAATTTTAC ATCATGCAAT TAAATTAGGA AAAGTGACAC ATATATTTAT
 2351 TACTCATATG CATGGCGATC ATATTTTGG TTGCCAGGA TTACTTTCTA
 2401 GTCGTTCTTT TCAGGGCGGT GAACAGAAGC CGCTTACATT GGTGGACCA
 2451 AAAGGAATTA AAGCATATGT GGAAATGTCT ATGAATTTAT CAGA

5

Mutant: NT6

10 **Phenotype:** temperature sensitivity

Sequence map: Mutant NT6 is complemented by plasmid pMP33, which contains a 2.3 kb insert of *S. aureus* genomic DNA. The partial restriction map of the insert is depicted in Fig. 23; open boxes along part of the length of the clone indicate the percentage of the clone for which DNA sequence has been obtained. Database searches at both the nucleic acid and protein levels reveal identity to the *S. aureus femA* gene, encoding a protein involved in peptidoglycan crosslinking (Genbank Accession No. M23918; published in Berger-Baechi, B., et al., *Mol. Gen. Genet.* 219, (1989) 263-269). The pMP33 clone contains the complete *femA* ORF (denoted in relative length and direction by an arrow) as well as 5' and 3' flanking DNA sequences, suggesting that it is capable to direct expression of the FemA protein.

25

DNA sequence data: The following DNA sequence represents sequence data acquired from subclones 1006, 1007 and 1008, using standard sequencing methods and the commercially-available primers T7 and SP6:

30

subclone 1006, a 500 bp Hind III fragment
SEQ ID NO. 4

1006.sp6 Length: 400 nt

35

1 AAATAATCTA AAAATTGGTA GTNCTCCTTC AGATAAAAAT CTTACTTTAA
 51 CACCATTCTT TTNAACTNNT TCCGTGTTTC TTTTCTAAG TCCATCCATA
 101 TTTTNAATGA TGTCATCTGC TGTTTTATCT TTTAAATCTA AACTGAGTG
 151 ATAACGGATT TGTAGCACAG GATCAAATCC TTTATGGAAT CCAGTATGTT
 201 CAAATCCTAA GTTACTCATT TTATCAAAGA ACCAATCATT ACCAGCATT
 40 251 CCTGTAATCT CGCCATCATG ATTCAAGTAT TGATATGGTA AATATGGATC
 301 GNTATGTAGG TATAGNCAAC GATGTTTTTT AACATATTTT GGATAATTCA
 351 TTAAAGNAAA AGTGTACGAG TNCTTGATTT TCATANTCAA TCACTGGACC

subclone 1007, a 900 bp Hind III fragment
SEQ ID NO. 5

45

1007.sp6 Length: 398 nt

```

1   TGC GTGAAAT NACTGTATGG CNTGCNATCT GTAAAGGCAC CAAACTCTTT
51  AGCTGT TAAA TTTGTAAACT TCATTATCAT TACTCCTATT TGTCTCTCGT
5   101 TAATTAATTT CATTTC CGTA TTTGCAGTTT TCCTATTTCC CCTCTGCAAA
151 TGTCAAAAAT AATAAATCTA ATCTAAATAA GTATACAATA GTTAATGTTA
201 AAACTAAAAC ATAAACGCTT TAATTGCGTA TACTTTTATA GTAATATTTA
251 GATTTTNGAN TACAATTTCA AAAAAAGTAA TATGANCGTT TGGGTTTGCN
301 CATATTACTT TTTTNGAAAT TGTATTCAAT NTTATAATTC ACCGTTTTTC
10  351 ACTTTTNCA AACAGTATTC GCCTANTTTT TTAAATCAA GTAAACTT

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subclone 1008, a 900 bp Hind III fragment
SEQ ID NO. 6

15 1008.sp6 Length: 410 nt

```

1   GTAATGACAA ATNTAACTAC AATCGCTTAA AATATTACAA AGACCGTGTG
51  TNAGTACCTT TAGCGTATAT CAACTTTAAT GAATATATTA AAGAACTAAA
101 CGAAGAGCGT GATATTTTAA ATAAAGATTT AAATAAAGCG TTAAAGGATA
151 TTGAAAAACG TCCTGAAAAT AAAAAAGCAC ATAACAAGCG AGATAACTTA
20  201 CAACAACAAC TTGATGCAAA TGAGCAAAAG ATTGAAGAAG GTAAACGTCT
251 ACAAGANGAA CATGGTAATG AATTACCTAT CTCTNCTGGT TTCTNCTTTA
301 TCAATCCATT TGANGTTGTT TATTATGCTG GTGGTACATC AAATGCATTC
351 CGTCATTTTN CCGGAAGTTA TGCAGTGCAA TGGGAAATGA TTAATTATGC
401 ATTAAATCAT

```

Mutant: NT8

Phenotype: temperature sensitivity

30 Sequence map: Mutant NT8 is complemented by plasmid pMP34,
which contains a 3.5 kb insert of *S. aureus* genomic DNA.
The partial restriction map of the insert is depicted in
Fig. 24. Database searches at both the nucleic acid and
protein levels reveal identity to the DNA sequence for the
35 *dfrB* (dihydrofolate reductase [EC 1.5.1.3]; EMBL entry
Z16422, published in Dale, G.E. et al. *Antimicrob. Agents
Chemother.* 37 (1993) 1400-1405) and *tysY* (thymidylate
synthase [EC 2.1.1.45]; EMBL entry X13290, published in
Rouch, D.A. et al. *Mol. Microbiol.* 3 (1989) 161-175) genes
40 of *S. aureus*. The relative size and orientations of the
genes, along with sequence identities, are depicted as
arrows in the restriction map:

DNA sequence data: The following DNA sequence represents
45 data acquired from clone pMP34, starting with M13 forward

and M13 reverse primers and applying primer walking strategies to complete the contig:

clone pMP34

5 SEQ ID NO. 7

pMP34 Length: 3479 nt

```

10      1  AAGCTTCATT AAAAAGTTTC TTCAATTTAT CAACATATTC AATGACGTTA
      51  GCATGTGCGA CACCAACGGA YTKSAKKTCA TGATCTCCTA TAAATTCAGC
      101 AATTTCCCTT TTCAAGTATT GGATACTAGA ATTTTGAGTT CTCGCATTGT
      151 GCACAAGCTC TAAGCGACCA TCATCTAGTG TACCAATTGG TTTAATTTTC
      201 ATAAGATTAC CAATCAAACC TTTTGTTTTA CTAATTCTGC CACCTTTAAT
      251 TAATTGATTC AATTGCCCTA TAACTACAAA TAATTTAATG TTTTCTCTTA
      15  301 AATGATTTAA CTTTTTAAGT ATTTTCAGAA TTGAGACACC TTCTTTTACA
      351 AGCTCTACTA GGTGTTGTAT TTGATACCCT AAACCAAAAG AAATAGATTT
      401 TGAATCAATA ACAGTTACAT TAGCATCTAC CATTTGACTT GCTTGGTAAG
      451 CAGTGTTATA TGTACCACTT AATCCTGAAG AAAGATGAAT ACTTATGATT
      501 TCAGAGCCAT CTTTCCCTAG TTCTTCATAA GCAGATATAA ATTCACCTAT
      20  551 GGCTGGCTGA CTTGTCTTTA CATCTTCATC ATTTTCAATA TGATTAATAA
      601 ATTCTTCTGA TGTAATATCT ACTTGGTCAA CGTATGAAGC TCCTTCAATA
      651 GTTAAACTTA AAGGAATTAC ATGWATGTTG TTTGCTTCTA ARTATTCTTT
      701 AGATAAATCG GATGTTGAGT CTGTTACTAT AATCTGTTTT GTCATGGTCC
      751 TTTTCCCCCT TATTTTTTAC GAATTAAATG TAGAAAGGTA TGTGGAATTG
      25  801 TATTTTTCTC ATCTAGTTTA CCTTCAACTG AAGAGGCAAC TTCCCAGTCT
      851 TCAAATGTAT AAGGTGGAAA GAACGTATCA CCACGGAATT TACCTTCAAT
      901 AACAGTAATA TACATGTCGT CCACTTTATC AATCATTCTT TCAAATAATG
      951 TTTGCCCTCC AAATATGAAA ACATGGCCCG GTAGTTGGTA AATATCTTCA
      1001 ATAGARTGAA TTACATCAAC GCCCTCTACG TTGAAACTTG TATCTGAAGT
      30  1051 AAGTACAACA TTTGACGAT TCGGTAGTGG TTTACCAATC GATTCAAATG
      1101 TCTTACGACC CATTACTAAA GTATGACCTG TTGATAATTT TTTAACATGC
      1151 TTCAAATCAT TTGGTAGGTG CCAAGGTAAT TGATTTTCAA AACCAATTAC
      1201 TCGTTGCAAG TCATGTGCAA CTAGAATGGA TAAAGTCATA ATTATCCTCC
      1251 TTCTTCTATC ATTTCAATTT TTATTACTAA GTTATCTTTA ATTTAACACA
      35  1301 ATTTTTATCA TAAAGTGTGA TAGAAATAAT GATTTTGCAT AATTTATGAA
      1351 AACGTTTAA ACAAAAAAGT ACTTTTTTGC ACTTGAAAAT ACTATGATGT
      1401 CATTTKGATG TCTATATGGT TAGCTAAYTA TGCAATGACT ACAMTGCTAT
      1451 KGGAGCTTTT ATKGCTGGAT GTGATTCATA GTCAACAATT TCCAMAATCT
      1501 TCATAATTTA TGTCGAAAAT AGACTTGTCA CTGTTAATTT TTAATGTTGG
      40  1551 AGGATTGAAG CTTTCACGTG CTAATGGTGT TKCGMATCGC ATCAATATGA
      1601 TTTGAATAAA TATGTGCATC TCCAAATGTA TGCACAAATT CACCCACTTC
      1651 AAGTCCACAT TTCTTTGGCA ATAAGGTGTG TCAATAAAGC GTAGCYTGCG
      1701 ATATTAAATG GCACACCTAA AAAGATATCT GCGCTACGTT GGTATAACTG
      1751 GCAACTTAAC TTACCATCTT GGACATAAAA CTGGAACATG GTATGACAAG
      45  1801 GCGGAAGTGC CATTGTATCA ATTTCTGTTG GATTCCATGC AGATACGATG
      1851 TGTCGCCTTG AATCTGGATT ATGCTTAATT TGTTC AATTA CTGTTTTAAG
      1901 TTGATCAAAA TGATTACCAT CTTTATCAAC CCAATCTCGC CMATTGTTTA
      1951 CCATAAACAT TTCCTAAATC CCCGAATTGC TTCGCAAATG TATCATCTTC
      2001 AAGAATACGT TGCTTAAAT GTTTCATTTG TTCTTTATAT TGTTGTTTAA
      50  2051 ATTCAGGATC ACTCAATGCA CGATGCCCGA AATCTGTCAT ATCTGGACCT

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2101 TTATACTCGT CTGATTTGAT ATAATTTTCA AAAGCCCATT CGTTCCATAT
2151 ATTATTATTA TATTTTAATA AGTATTGGAT GTTTGTATCT CCTTTAATGA
2201 ACCATAATAA TTCGGTTGCT ACTAATTTAA AAGAACTTT CTTTGTCTGT
2251 AATAGTGGAA ATCCTTTAGA TAAGTCAAAG CGAAGTTGAT GACCAAATTT
5 2301 CGAAATCGTA CCTGTATTTG TGCGATCATT TCGTGTATTT CCTATTTCTA
2351 AAACCTCTTC ACAAGACTG TGATATGCTG CATCAAATGA ATTTCAACAT
2401 ATGCGATAAC ACCTCATTTT CATTATTTAT AGTATGTATA TTTAGTTTGA
2451 TATAACTTAA CTTTATGTAG CATTTTGTTA TCACTCATTT TAGGAATATG
2501 ATATTAATAT CATGAATTCC GTTACTTTAT TTATAAAATG CTGATTAAGT
10 2551 ACCTACCCCA TCGTAACGTG ATATATGTTT CCAATTGGTA ATTGTTTACC
2601 CAAATCTATA ACTTTAATGC TAAAAAATTT TAAAAAAGAG GTTAACACAT
2651 GATTGAATA TTATGTTTGA TGTCCTATTA AAACAGTTAA ATTTCTAGAA
2701 AATATAGTTG GTAAAAACGG ACTTTATTTA ACAAAATAGAA TACAACTATA
2751 TTCTCTATTT TCAATGACAG ACACCATTTT TAATATTATA AAATGTGTTA
15 2801 ACCTTTATAT TTATTTATGT GTACTATTTA CAATTTTCGT CAAAGGCATC
2851 CTTTAAGTCC ATTGCAATGT CATTAATATC TCTACCTTCG ATAAATTCTC
2901 TAGGCATAAA ATAAACTAAA TCTTGACCTT TGAATAAAGC ATACGAAGGA
2951 CTAGATGGTG CTTGCTGAAT GAATTCTCGC ATTGTAGCAG TTGCTTCTTT
3001 ATCTTGCCCA GCAAAAAC TGTAAGTATT TGTAAGTCTA TGTTTCAATTT
20 3051 GTGTTGCAAC TGCTACTGCA GCTGGTCTTG CTAATCCAGC TGCACAGCCG
3101 CATGTAGAGT TAATAACTAC AAAAGTAGTG TCATCAGCAT TTACTTGGTT
3151 CATATACTCC GATACTGCTT CGCTCGTTTC TAAACTTGTA AAACCATTTT
3201 GAGTTAATTC GCCACGCATT TGTGCGCAA TTTCTTTCAT ATAAGCATCA
3251 TAYGCATTCA TATTTAATTC CTCCAATTAA ATTGTTCTGT TTGCCATTTG
25 3301 TYTCCATACT GAACCAAGYG CTTCACTCTC GTTTTCAATA TCGAGATATG
3351 GCCATTTCAT TTTGTAATTT AACWTCAAAC GCMTKGTC AKATATGGGS
3401 WTTTAGKGC GGAAGMTGMT YWGCATWACS WTCATSAWAG ATAWACAYAG
3451 CARCAYSCCA CYTWAYGAKT TTMWKTGGA

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30

Mutant: NT12

Phenotype: temperature sensitivity

35 Sequence map: Mutant NT12 is complemented by pMP37, which contains a 2.9 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 25. Database searches at both the nucleic acid and peptide levels reveal significant similarities to the protein encoded by the *tagG* gene, an integral membrane protein involved in the assembly of teichoic acid-based structures, from *B. subtilis* (Genbank Accession No. U13832; published in Lazarevic, et al., *Mol. Microbiology*, 16 (1995) 345-355).

45 DNA sequence data: The following DNA sequence data represents the sequence of clone pMP37, using standard M13 forward and M13 reverse sequencing primers and then

completing the sequence contig via primer walking strategies. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

5

clone pMP37

SEQ ID NO. 8

pMP37 Length: 2875 nt

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1  GTGGTTCCTT GTCATTYTRA TATCCATCAA ACCTTTATTA ATACACGTRG
51  CTATCGAAGC ATTTTGTAAT TGTATTAATG AAATATGCTT GAGTYCTCTT
101 TGTAACCGTT CAATCATAGG AATTGTTTGA TCAGTAGAAC CACCATCAAT
151 ACAAAGGATT CTATAGTGTT CTTTACTCTC AATAGATATT AACAAATTGTC
201 GAATTGTTGC CTCATTATTA CATGTAGGTA TGATTATCGT AAACCTCATT
251 TTGTCACCAT CTTATCTATA TATTCTGTGA GCTGATGTAA ACTTTTATCA
301 GTATTATACT TATGCCAATC TTAAATAAAC GGACTTAATA GATGTTCTTT
351 TTCTTGATC GTCATTATTA AATCTTCTTC AGTATACACT TTGTAGCTAT
401 CCGGTATTGC TTTGTAAAAT TGATTGAGG CTCTCACCTG ATCATATGTT
451 CCTTCATCAT ACACATAAAA TATAGTTGGA ATATCTAACA AGCTAGCTTC
501 TATTGGCAGC GAACTATAGT CGCTAATAAT TATATCTGAC ATTAGCATT
551 ATGTAGACGT GTCGATTGAA GATACGTCAT CAATGTCTGA ATCTTCAATT
601 GATGGATGTA ATTTATTAAT CAGTGTATAT CCTGGTAAAC ATTTTTCAAA
651 ATAAGCTTTA TCAATAGCCC TATTATCTGC TTTATCTTCT CTATATGTTG
701 GTACATATAA TACCAACTTA TTTGTAATTC CATATTTATC CTTTAACTCT
751 GCCTTAACCG TTGCTCTATC AGCTGTGTAA TATTTATTAA TTCTCGGAAG
801 CCCAAAATAC AGCATTGCTT CTTCTGTTGC ACCTAAAGAC TGTTTTAAAC
851 ATTGTGACAT TTGTTCAACA CCCACTAAGT TAAAAATCCG TCGCTTGATA
901 AACTTTACCG TACTGCTGAA CCATTGCCTT GTCAGACACA TCGACTTGAT
951 GATCTGTTAA GCCAAAGTTT TTTAATGCAC CACTTGCATG CCACGTTTGA
1001 ACAATGTGTT TGATTAGAAK TCTTATTATA TCCACCTAGC MATAGGTAAT
1051 AATTATCGAT AATAATCATC TGC GCGCTTT TCAAAGCCTT AATTGTTTTT
1101 ACCAATGTTT GATTAGTCAT TTCTATCACA TCAACATCGT CGCTAAGTTC
1151 AGATAAATAA GGCGCTTGTT TTGGTGTGTG TAAAACAGTT TTCTGATACG
1201 ACGAATTATT TAATGCTTTG ATGATAGGCT TAATATCTTC TGGAAAAGTC
1251 ATCATAAATA CGATATGCGG TTTATCAATC ACTTGAGGSG TAWTCATTTW
1301 AGRAAGTATT CGAACTACCA AATGATAAAA TTTCTTTATT AAAAACGTTT
1351 ATAATAACAC CAACTTAATA TGTTATTTAA CTAAATTAT AAACAAAAT
1401 GAACCCCACT TCCATTTATT AATGGTTAGC GGGGTTTCGT CATATAAATA
1451 TATTACAAGA AGTCTGCAAA TTGATCTCTA TATTTATGAT GTWAGTACGC
1501 MCCMATGCA AAGAAAATGG CAACAATACC GAAATTGTAT AACATTAATT
1551 TCCAATGATC CATGAAATAC CATTCGTGAT ATAAAATTGC TGCACKKTWT
1601 KATMAKCWR TAMRGTMAC TRGMTKATAT TTCATCATTK SATGAATTAA
1651 ACCACTGATA CCATGGTTCT TTGGTAGCCA CAAAATTGGT GAAAAGTAAA
1701 ATAATATTCT TAATATTGGC TTGCATTAAC ATTTGTGTAT CTCTAACTAA
1751 CAACACCGAG TGTTGATGTT AATAACGTC CCGAGGCAGT TAAGAAAAAA
1801 CAAAACGGTA CATATATCAA TAATTGAATG ATATGTATTG ATGGATAAAT
1851 ACCAGTAAAC ATACATGCAA TTATCACAAG TAAAAGTAAG CCTAAATGTC
1901 CATAAAATCT ACTTGTGACA ATATATGTCG GTATTATCGA TAACGGGAAG
1951 TTCATTTTCG ATACTTGATT AAACCTTTGT GTAATTGCTT TAGTACCTTC
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2001 TAAAATACCT TGGTTGATGA AGAACCACAT ACTGATACCA ACCAATAACC
 2051 AATAAACAAA AGGTACACCA TGAATTGGTG CATTACTTCT TATTCCTAAT
 2101 CCAAAAACCA TCCAGTAAAC CATAATTTGC ATAACAGGGT TAATTAATTC
 2151 CCAAGCCACA CCTAAATAGT TACTATGATT GATAATTTTA ACTTGAAACT
 5 2201 GAGCCAGTCT TTGAATTAAA TAAAAGTTCT WTASATGTTT TTTAAAAACT
 2251 GTTCCTATTG CTGACATTCC ATTAAACCAC ACTTTCAAAT GTTTAACTAT
 2301 TTCTCTAACT TAACTAAATA GTATTATAAT AATTGTTGTA AATACTATCA
 2351 CTAWACATGG ATGCTATCAA AATTATTGTC TAGTTCTTTA AAATATTAGT
 2401 TTATTACAAA TACATTATAG TATACAATCA TGTAAGTTGA AATAAGTTTA
 10 2451 GTTTTTTAAAT ATCATTGTTA TCATTGATGA TTAACATTTT GTGTCAAAAC
 2501 ACCCACTCTG ATAATAACAA AATCTTCTAT ACACTTTACA ACAGGTTTTA
 2551 AAATTTAACA ACTGTTGAGT AGTATATTAT AATCTAGATA AATGTGAATA
 2601 AGGAAGGTCT ACAAATGAAC GTTTCGGTAA ACATTAAAAA TGTAACAAAAA
 2651 GAATATCGTA TTTATCGTAC AAATAAAGAA CGTATGAAAG ATGCGCTCAT
 15 2701 TCCCAAACAT AAAAAACAAA CATTTTTTCGC TTTAGATGAC ATTAGTTTAA
 2751 AAGCATATGA AGGTGACGTC ATAGGGCTTG TTGGCATCAA TGGTTCCGGC
 2801 AAATCAACGT TGAGCAATAT CATTTGGCGG TCTTTGTCGC CTACTGTTGG
 2851 CAAAGTGGAT CGACCTGCAG TCATA

20

Mutant: NT14

Phenotype: temperature sensitivity

25 **Sequence map:** Mutant NT14 is complemented by plasmid
 pMP40, which contains a 2.3 kb insert of *S. aureus* genomic
 DNA. The partial restriction map of the insert is depicted
 in Fig. 26 (no Eco RI, Hind III, Bam HI or Pst I sites are
 apparent); open boxes along part of the length of the clone
 30 indicate the percentage of the clone for which DNA sequence
 has been obtained. Database searches at both the nucleic
 acid and protein levels reveal identity to the *Staph.*
aureus femB gene, encoding a protein involved in
 peptidoglycan crosslinking (Genbank Accession No. M23918;
 35 published in Berger-Baechi, B., et al., Mol. Gen. Genet.
 219, (1989) 263-269). The pMP40 clone contains the
 complete FemB ORF (denoted in relative length and direction
 by an arrow) as well as 5' and 3' flanking DNA sequences,
 suggesting that it is capable to direct expression of the
 40 FemB protein; the relation of the femA gene is also
 depicted to demonstrate the extent of identity between the
 clone and the Genbank entry.

DNA sequence data: The following DNA sequence data
 45 represents the sequences at the left-most and right-most

edges of clone pMP40 obtained with the standard DNA sequencing primers T7 and SP6, and can be used to demonstrate identity to part of the published sequence (Genbank No. M23918):

5

SEQ ID NO. 9

1015.t7 LENGTH: 453 nt

1 CTTAAAATAT TACAAAGACC GTGTGTNAGT ACCTTNAGCG TATATcAaCT
 51 TTAATGAATA TATTAAAGAA CTAAACGAAG AGCGTGATAT TTTAAATAAA
 10 101 GATTTAAATA AAGCGTTAAA GGATATTGAA AAACGTCCTG AAAATAAAAA
 151 AGCACATAAC AAGCGAGATA ACTTACAACA ACAACTTGAT GCAAATgAGC
 201 AAAAGATTGA NGACGGTAAA CGTCTACAAG ANGANCATGG TAATGNTTTA
 251 CCTATCTCTC CTGGTTTCTC CTTTATCAAT CCNTTTGANG TTGTTTATTA
 301 TGCTGGTGGT ACATCAAATG CNTTCCGTCA TTTTNC CGGA NGTTATGCNG
 15 351 TGCAATGGGA AATGNTTAAT TTTGCATTAA ATCATGGCAT TGNC CGTTAT
 401 AATTNCTATG GTGTTAGTGG TNAATTTNCA GNAGGTGCTG AAGATGCTGG
 451 TGT

SEQ ID NO. 10

20 1015.sp6 LENGTH: 445 nt

1 ATGCTCAGGT CGATCATACA TCTATCATCA TTtAATTTC TAAAATACAA
 51 ACTGAATACT TTCCTAGAA NTNaACAGC AATCATTGCT CATGCATTTA
 101 ATAAATTaCA ATTAGACAAA TATGACATT gATATCACAC ACTTGCAAAC
 151 ACACACATAT ATAATCAGAC ATAAATTGTT ATGCTAAGGT TTATTCACCA
 25 201 AAANTATAAT ACATATTGGC TTGTTTGGAG TCATATTGNN TGANTTANAA
 251 NGTATCTCA ACTCANTCAT TTNCAAATNG GTTGTGCAAT TCNTATTTNT
 301 NTTTCTTGCA ATCCCTTGTT AACTTGTC TTTNATATAT CATNTTCGG
 351 GGCTTTATTA AAANNCA TNNACNGNGC CTATNGNNTC NNTNACTATN
 401 NGCCCTAACA TCATTTTCNT CTNTTCTTA TTTTACGG GATT

30

Mutant: NT15

Phenotype: temperature sensitivity

35 Sequence map: Mutant NT15 is complemented by plasmid pMP102, which contains a 3.1 kb insert of *S. aureus* genomic DNA. The partial restriction map of the insert is depicted in Fig.27; open boxes along part of the length of the clone indicate the percentage of the clone for which DNA sequence
 40 has been obtained. Database searches at both the nucleic acid and protein levels reveal strong identity at both the peptide and nucleic acid level to the SecA protein from *S. carnosus* (Genbank Accession No. X79725; submitted in 1994, unpublished as of 1995); the relative size and location of
 45 the *secA* gene predicted from similarity to the *S. carnosus* gene is depicted below by an arrow. The SecA protein is

involved in the protein secretory pathway and serves an essential cellular function.

DNA sequence data:

5 clone pMP102
SEQ ID NO. 11

pMP102.forward Length: 719 nt

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10      1  GATCRAGGAG ATCAAGAAGT GTTTGTGGCC GAATTACAAG AAATGCAAGA
      51  AACACAAGTT GATAATGACG CTTACGATGA TAACGAGATA GAAATTATTC
     101  GTTCAAAAGA ATTCAGCTTA AAACCAATGG ATTCAGAAGA AGCGGTATTA
     151  CAAATGAATC TATTAGGTCA TGACTTCTTT GTATTCACAG ACAGAGAAAC
     201  TGATGGAACA AGTATCGTTT ACCGCCGTAA AGACGGTAAA TATGGCTTGA
     15  251  TTCAAAC TAGAACAATAA ATTAAGTTTA AAGCACTTGT GTTTTTCAC
     301  AAGTGCTTTT TTATACTCCA AAAGCAAATT ATGACTATTT CATAGTTCGA
     351  TAATGTAATT TGTTGAATGA AACATAGTGA CTATGCTAAT GTTAATGGAT
     401  GTATATATTT GAATGTTAAG TTAATAATAG TATGTCAGTC TATTGTATAG
     451  TCCGAGTTCG AAAATCGTAA AATATTTATA ATATAATTTA TTAGGAAGTT
     20  501  ATAATTGCGT ATTGAGAATA TATTTATTAG TGATAAACTT GTTTGACACA
     551  GAATGTTGAA TGAATTATGT CATAAATATA TTTATATTGA TCTACCAATG
     601  AGTAAATAAN TATAATTTCC TAACTATAAA TGATAAGANA TATGTTGTNG
     651  GCCCAACAGT TTTTGCTAA AGGANCGAAC GAATGGGATT TTATCCAAAA
     701  TCCTGATGGC ATAATAAGA

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25

SEQ ID NO. 12

pMP102.reverse Length: 949 nt

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      1  CTTTACCATC TTCAGCTGAA ACGTGCTTCG CTTACCAAAA CTCTGTTGTT
     30  51  TTTTCACGTT CAATATTATC TTCAACTTGT ACTACAGATT TTAATGAA
    101  TTTACAAGTA TCTTCTTCAA TATTTTGCAT CATGATATCA AATAATTCAT
    151  GACCTTCATT TTGATAGTCA CGTAATGGAT TTTGTTGTGC ATAAGAACGT
    201  AAGTGAATAC CTTGACGTAA TTGATCCATT GTGTCGATAT GATCAGTCCA
    251  ATGGCTATCA ATAGAACGAA GTAAAATCAT ACGCTCAAAC TCATTCATTT
    35  301  GTTCTTCTAA GATATCTTTT TGACTTTGAT ATGCTGCTTC AATCTTAGCC
    351  CAAACGACTT CGAAAATATC TTCAGCATCT TTACCTTTGA TATCATCCTC
    401  TGTAATGTCA CCTTCTTGTA AGAAGATGTC ATTAATGTAG TCGATGAATG
    451  GTTGATATTC AGGCTCGTCA TCTGCTGTAT TAATATAGTA ATTGATACTA
    501  CGTTGTAACG TTGAACGTAG CATTGCATCT ACAACTTGAG AGCTGTCTTC
    40  551  TTCATCAATA ATACTATTTT TTTTCGTTATA GATAATTTCA CGTTGTTTAC
    601  GTAATACTTC ATCGTATTCT AAGATACGTT TACGCGCGTC GAAGTTATTA
    651  CCTTCTACAC GTTTTTGTGC TGATTCTACA GCTCTTGATA CCATTTTTGA
    701  TTCAATTGGT GTAGAGTCAT CTAAACCTAG TCGGCTCATC ATTTTCTGTA
    751  AACGTTTCTA ACCAAAACGA AATCATTAAT TCATCTTGTA ATGATAAATA
    45  801  GAAGCGACTA TCCCTTTTAT CACCTTGACG TCCAGAACGA CCACGTAAC
    851  GGTCATCAAT ACGACGAAGA TTCATGTCGC TCTGTACCTA TTAGTCTAA
    901  ACCGCCTAAT TCCTCTACGC CTTACCTAA TTTGATATCT GTACCACGA

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SEQ ID NO. 13

pMP102.subclone Length: 594 nt

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      1  GGGGATCAAT TTANAGGACG TACAATGCCA GGCCGTCGTT NCTCGGAAGG
5      51  TTTACACCAA GCTATTGAAG CGAGGAAAGG CGTTCAAATT CAAAATGAAA
      101  TCTAAAACTA TGGCGTCTAT TACATTCCAA AACTATTTC AATGTACAA
      151  TAAACTTGCG GGTATGACAG GTACAGCTAA AACTGAAGAA GAAGAATTTA
      201  GAAATATTTA TAACATGACA GTAACTCAA TTCCGACAAA TAAACCTGTG
      251  CAACGTAACG ATAAGTCTGA TTTAATTTAC ATTAGCCAAA AAGGTAAATT
10     301  TGATGCAGTA GTAGAAGATG TTGTTGAAAA ACACAAGGCA GGGCAACCMG
      351  TGCTATTAGG TACTGTTGCA GTTGAGACTT CTGTATATAT TTCAAATTTA
      401  CTTAAAAAAC GTGGTATCCG TCATGATGTG TTAAATGCGA RAAATCATGA
      451  MCGTGAAGCT GAAATTGTTG CAGGCGCTGG RCAAAAAGGT GCCGTTACTA
      501  TTGCCACTAM CATGGCTGGT CGTGGTACAG ATATCAAATT AGGTGAAGGC
15     551  GTTANAANGA AATTAGGCGG TTTANCCAGT AATANGTTCA GAAG

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Mutant: NT1620 **Phenotype:** temperature sensitivity

Sequence map: Mutant NT16 is complemented by plasmid pMP44, which contains a 2.2 kb insert of *S. aureus* genomic DNA. The partial restriction map of the insert is depicted in Fig. 28. Database searches at both the nucleic acid and protein levels reveal significant similarity at the peptide level to an ORF (orf3) of unknown function in the serotype "A" capsulation locus of *H. influenzae* (Genbank Accession No. Z37516); similarity also exists at the protein level to the tagB gene of *B. subtilis* (Genbank Accession No. X15200), which is involved in teichoic acid biosynthesis. Based upon the peptide level similarities noted, it is possible that the ORF(s) contained within this clone are involved in some aspect of membrane biogenesis, and should make an excellent screening target for drug development.

35 No significant similarities are observed at the nucleic acid level, strengthening the stance that clone pMP44 represents a novel gene target(s).

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP44, starting with standard M13 forward and M13 reverse sequencing primers. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

45

clone pMP44
SEQ ID NO. 14

pMP44 Length: 2192 nt

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5
1 GCATGMCTGC AGGTCGATCY SYTGAACAGT CATCAACTAC AACCACCTTCA
51 AATTCAGTTT TCGGAAAATC TTGTTTCGCA AGGCTATTAA GTAATTCTGT
101 TATATACTTT TCTGAATTGT ATGTTTGAAC TATTACTGAA AATTTTCATCA
151 TTATACCTCT CCCACTTTGA CTACTATATA AACTTAGCTA CCAAATAAAT
10 201 TTCTGACTAA ACGCTCACTT GATCGGCCAT CTTGATATTT AAAATGTTTA
251 TCTAAGAATG GAATGACTTT TTCTCCTTCA TAATCTTCAT TGTCCAAGGC
301 GTCCATTAAT GCGTCAAATG ATTGCACAAT TTTACCTGGA ACAAAATGATT
351 CATATGGTTC ATAAAAATCA CGCGTCGTAA TATAATCTTC TAAATCAAAT
401 GCATAGAAAA TCATTGGCTT TTTAAATACT GCATATTCAT ATATTAAAGA
15 451 TGAATAGTCA CTAATTAATA AATCTGTTAT GAACAGTATA TCATTAACCTT
501 CTCTAAAGTC AGAAACGTCA ACAAATATT GTTTATGTTT GTCTGCAATA
551 TTAAGTCTAT TTTTCACAAA TGGATGCATT TTAAATAATA CAACCGCGTT
601 ATTTTTTTTCG CAATATCTTG CTAAACGTTT AAAATCAATT TTGAAAAATG
651 GGTAATGTGC TGTACCATGA CCACTACCTC TAAATGTTGG TGCGAAAAGA
20 701 ATGACTTTCT TACCTTTAAT AATTGGTAAT TCATCTTCCA TCTCTTGTTT
751 GATCTGTGTC GCATAAGCTT CATCAAATAG TACATCAGTA CGTTGGGAAC
801 ACCTGTAGGC ACTACATTTT TCTCTTTAAT ACCAAATGCT TCAGCGTAGA
851 ATGGAATATC GGTTTCAAGA TGATACATAA GCTTTTGTAT AAGCTACGGA
901 TGATTTAATG AATCAATAAA TGGTCCACCC TTTTACCAG TACGACTAAA
25 951 GCCAACTGTT TTAAAGGCAC CAACGGCATG CCATACTTGA ATAACTTCTT
1001 GAGAACGTCT AAAACGCACT GTATAAATCA ATGGGTGAAA GTCATCAACA
1051 AAGATGTAGT CTGCCTTCCC AAGTAAATAT GGCAATCTAA ACTTGTCGAT
1101 GATGCCACGT CTATCTGTAA TATTCGCTTT AAAAACAGTG TGAATATCAT
1151 ACTTTTTTATC TAAATTTTGA CGTAACATTT CGTTATAGAT GTATTCAAAG
30 1201 TTTCCAGACA TCGTTGGTCT AGAGTCTGAT GTGAACAACA CCGTATTCCC
1251 TTTTTTCAAG TGGAAAAATT TCGTCGTATT AAATATCGCT TTAAAAATAA
1301 ATTGTCTTGT ATTAAATGAT TGTTTGCGGA AATACTTACG TAATTCTTTA
1351 TATTTACGRA CGATATAAAT ACTTTTAAMT TCCCGGAGTC GTTACAACAA
1401 CATCAAGGAC AAATTCATTA ACATCGCTAG AAATTTCAGG TGTAACAGTA
35 1451 TAAACCGTTT TCTTTTCGAA TGCCGCCTTT TCTAAATTCT TTTAGGTAAG
1501 TCTGCAATAA GAAATTGATT TTACCATTTT GTGTTTCTAA TTCGYTGTAT
1551 TCTTCTTCTT GTTCTGGCTT TAGATTTTGA TATGCATCAT TAATCAACAT
1601 CTGGGTTTAA CTGTGCAATA TAATCAAGTT CTTGCTCATT CACTAATAAG
1651 TACTTATCTT CAGGTAAGTA ATAACCATTA TCTAAGATAG CTACATTGAA
40 1701 ACGACAAACG AATTGATTCC CATCTATTTT GACATCATTC GCCTTCATTG
1751 TACGTGTCTC AGTTAAATTT CTTAATACAA AATTACTATC TTCTAAATCT
1801 AGGTTTTTCAC TATGTCCTTC AACGAATAAC TGAACACGTT CCCAATAGAT
1851 TTTAYCTATA TATATCTTAC TTTTAACCAA CGTTAATTCA TCCTTTTCTA
1901 TTTACATAAT CCATTTTAAAT ACTGTTTTAC CCCAAGATGT AGACAGGTCT
45 1951 GCTTCAAAAG CTTCTGTAAG ATCATTAAAT GTTGCAATTT CAAATTCCTG
2001 ACCTTTTAAA CAACGGCTAA TTTATCTAAC AATATCTGGG TATTGAATGT
2051 ATAAGTCTAA CAACATCTTG GAAATCTTTT GAACCACTTC GACTACTACC
2101 AATCAACGTT AGTCCTTTTT CCAATACTAG AACGTGTATT AACTTCTACT
50 2151 GGGAACTCAC TTACACCTAA CAGTGCAATG CTTCTTCTG GT
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Mutant: NT17

Phenotype: temperature sensitivity

5 **Sequence map:** Mutant NT17 is complemented by plasmid
pMP45, which contains a 2.4 kb insert of *S. aureus* genomic
DNA. The partial restriction map of the insert is depicted
in Fig. 29. Database searches at both the nucleic acid and
protein levels reveal a strong similarity to the product of
10 the *apt* gene, encoding adenine phosphoribosyl transferase
(EC 2.4.2.7) from *E. coli* (Genbank Accession No. M14040;
published in Hershey, H.V. et al. *Gene* 43 (1986) 287-293).

DNA sequence data: The following DNA sequence data
15 represents the sequence generated by primer walking into
clone pMP45, starting with standard M13 forward and M13
reverse sequencing primers. The sequence below can be used
to design PCR primers for the purpose of amplification from
genomic DNA with subsequent DNA sequencing:

20

clone pMP45
SEQ ID NO. 15

25

pMP45 Length: 2431 nt

30

35

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45

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1  ATGCAGGTCG ATCNCCTNGT TTATTCNGNT TCATCATTTT CCGATAAATA
51 CTGTAAATAT GNNTAGGTCT ACCATTTATA TCGCCTTCGA TATTCATTCTG
101 GTCCATTTCG GTACGTATTC TATCAATAGC CGTTTCGATA TACGCTTCAC
151 GTTCACTACG TTTCTTCTTC ATTAAATTGA CTATTCTAAA ATATTGCACA
201 TTATCAATAT AACGAAGAGC CGKATCTTCT AGTTCCCATT TGATTGTATT
251 AATACCAAGA CGATGTGCTA ATGGTGCATA AATTTCTAAT GTTTCTCGAG
301 AAATTCTAAT TTGKTTTTCG CGCGGSATGG STTTCAAGGT ACGCATATTA
351 TGTAATCTGT CTGCTAATTT CAMCAAAATT ACGCGTACAT CTTTGGCAAT
401 CGCAATAAAT AACTTGSGAT GATTTTCAGC TTGTTGTTCT TCTTTTGAGC
451 GGTATTTTAC TTTTSTAAGC TTCGTACACAC CATCAACAAT TCGAGCAACT
501 TCTTCATTGA ACATTTCTTT TACATCTTCA AATGTATACG GTGTATCTTC
551 AATTACATCA TGCAAAAAAC CTGCGACAAT CGTCGGTCCG TCTAATCGCA
601 TTTCTGTAA AATACCTGCA ACTTGTATAG GATGCATAAT GTATGGTAAT
651 CCGTTTTTTC GGAACGACC TTTATGTGCT TCATAAGCAA TATGATAGCT
701 TTTTAAAACA TACTCATATT CATCTGCTGA CAAATATGAT TTTGCTTTGT
751 GAAGAACTTC GTCTGCACTA TATGGATATT CGTTGTTTCT TATATGATAC
801 ACCCCATTCA TATTTATTAC TTCGCCTTTA AACAATGGAT TTAGGTACTC
851 TTGTTGAATA GTATTGTGCC CACACCAATC ATACGTCCGT CGACGATAAA
901 TATTTATCCT GTCGTGCATT AATCGTAATA TTAATTTTAC TTGAGCGAGT
951 TTAATTTGTA TACTATTCCT ACTTTTAAAA CTTTACAAA AATTCGACCT
1001 AAATCTACTG TTTCAATTTT TAAATATTAG TTCTATGATA CTACAATTTA
1051 TGARATAAAT AAACGAWGTT ATTAAGGTAT AATGCTCMAT CATCTATCAT

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1101 TTTTCAGTAAA TAAAAAATCC AACATCTCAT GTTAAGAAAA CTAAACAAC
1151 TTTTFTAATT AAATCATTGG TYCTTGWACA TTTGATRGAA GGATTTCATT
1201 TGATAAAATT ATATTATTTA TTATTGTCG TATGAGATTA AACTMATGGA
1251 CATYGTAATY TTAAAWAKTT TTCMAATACC AWTAAAWKA TTTCAATTCA
5 1301 AATTATAAAW GCCAATACCT AAYTACGATA CCCGCCTTAA TTTTTCAACT
1351 AATTKTATKG CTGYTCAATC GTACCACCAG TAGCTAATAA ATCATCTGTA
1401 ATRRSACAG TTGACCTGGK TTAATTGCAT CTTKGTGCAT TGTYAAAACA
1451 TTTGTACCAT ATTCTAGGTC ATAACTCATA ACGAATGACT TCACGAGGTA
1501 ATTTCCCTTC TTTTCTAACA GGTGCAAAGC CAATCCCCAT KGAATAAGCT
10 1551 ACAGGACAGC CAATGATAAA GCCAACGSGC TTCAGGTCCW ACAACGATAT
1601 CAAACATCTC TGTCTTTTGC GTATTWACA ATTTTATCTG TTGCATAGCC
1651 ATATGCTTCA CCATTATCCA TAATTGTAGT AATATCCTTG AAATAACAC
1701 CTGGTTTCGG CCAATCTTGA ACTTCTGATA CGTATTGCTT TAAATCCATT
1751 AATATTTCCCT CCTAAATTGC TCACGACAAT TGTGACTTTA TCCAATTTTT
15 1801 TATTTCTGAA AAATCTTGAT ATAATAATTG CTTTTCAACA TCCATACGTT
1851 GTTGTCTTAA TTGATATACT TTGCTGGAAT CAATCGATCT TTTATCAGGT
1901 TGTGATTGA TTCGAATTAA ACCATCTTCT TGTGTTACAA ATTTTAAGTC
1951 TAAGAAAAC TTCAACATGA ATTTAAGTGT ATCTGGTTTC AACTTAAAT
2001 GTTGACACAA TAACATACCC TCTTTCTGGA TATTTGTTTC TTGTTAGTT
20 2051 ATTAATGCTT TATAACACTT TTTAAAATA TCCATATTAG GTATACCATC
2101 GAAGTAAATC GAATGATTAT GTTGCAAAAC TATAKAAAGW TGAGAAAATT
2151 GCAGTTGTTG CAAGGAATTA GACAAGTCTT CCATTGACGT TGGTAAATCT
2201 CTTAATACTA CTTTATCAGT TTGTTGTTTA ATTTCTTCAC CATAATAATA
2251 TTCATTCGCA TTTACTTTAT CACTTTTAGG ATGAATAAGC ACGACAATAT
25 2301 TTTCATCATT TTCTGTAAAA GGTAAACTTT TCGCTTACT TCTATAATCT
2351 AATATTTGCT GTTCATTCAT CGCAATATCT TGAATAATTA TTTGCGGTGA
2401 TTGATTACCA TTCCATTCGT TGATTGAAC A

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30

Mutant: NT18

Phenotype: temperature sensitivity

Sequence map: Mutant NT18 is complemented by pMP48, which
35 contains a 4.7 kb insert of *S. aureus* genomic DNA. A
partial restriction map is depicted in Fig. 30, along with
open boxes to indicate the percentage of the clone for
which DNA sequence has been obtained; the sequence contig
will be completed shortly. Database searches at both the
40 nucleic acid and peptide levels reveal a strong peptide-
level similarity to the *ureD* gene product, encoding a
putative regulatory protein with strong similarities to the
phosphomannomutase and the phosphoglucomutase from *E. coli*.
The right-most sequence contig from the diagram below is
45 responsible for complementing mutant NT102, described
later; however, the full pMP48 clone described here is
required for complementing mutant NT18. Based upon genomic

organization and peptide-level similarities, it is highly likely that mutants NT18 and NT102 represent two different proteins in the same biochemical pathway.

- 5 **DNA sequence data:** The following DNA sequence data represents the sequence obtained from clone pMP48, starting with standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to augment the sequence contigs. The sequences below can be used to
10 design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP48
SEQ ID NO. 16

15

pMP48.forward Length: 2018 nt

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      1 GCATCAGTTG GTACTTTAAA TAAATGTGCA GTACCAGTCT TAGCAACATT
      51 TACAGTTGCT AATTCAGTAT TTTTCTTAGC ATCTTTAATA ACTAAATTTG
20    101 TTGCACCTTG CTTACTATTC GTTTGCATAG TAGTAAAGTT AATAATTAAT
      151 TCTGAATCTG GTTTTACATT TACAGTTTTT GAAATACCGT TAAAGTTACC
      201 ATGATCTGTA GAATCATTTG CATTACACAG ACCTAATGCA GCCACGTTTC
      251 CTTTAGCTTG ATAGTTTGA GGGTTATTCT TATCAAACAT ATCGCTTCGT
      301 CTTAATTCTG AGTTAACGAA ACCAATCTTA CCGTTGTTAA TTAATGAATA
25    351 ACCATTTACT TTATCTGTAA CAGTTACAGT TGGATCCTGT CTATTCTCAT
      401 CTGTTGATAT GGCAGGATCA TCAAATGTTA ATGTCGTATT AATACTGCCT
      451 TCACCAGTAT TGCTAGCATT TGGATCTTGA GTTTGTGCGT TTGCTGCTAC
      501 AGGTGCTGCT GGTGCGCTG CTGCTGGANC ATTCGCTGGC TGTGTTTGAT
      551 TTGCCGGTGT TGCATTATTA TWAGGTGTTG CTTGGTTATT TCCTTGACCT
30    601 GCTTGGTWTG CCGGTGTTGC TTGATTTCCA GGTGTGTCAT GTGCAACGTT
      651 ATTCGGATCA GCTTGATCAC CTTGTCCAGC TGGTTGTGTA TTTGGTTGTG
      701 CTGCTCCTCC TGCTGGATTA GCCTGTCCAC CTTGGTTTGC TGGTTGTACT
      751 GCTGGTTGTC CTTGGTTGGC AGGTGCAGCT GGCTGTGCTG TAGGATTAGC
      801 TTGAGCACCA GCATTTGCGT TAGGCTGTGT ATTGGCATCA GCTGGTTGTG
35    851 CTGGTTGATT TTGTGCAGGC TGATTTTGCT CTGCTGCAKA CGCTGTTGTC
      901 GGGTTAGTAG ATATAAAAGT AACAGTGGCA ATTAAAGCTG AAAAAATACC
      951 GACATTAAAT TTTCTGATAC TAAATTTTTG TTGTCTGAAT AAATTCATTA
     1001 AGTCATCCTC CTGGTTGATT ATTCTCGCTG TTAAATGATT TCACTTAATC
     1051 AACTGTTAAG ATAAGTAGTA GCATCTGCGT TAAAAACACA AAGCAACTCT
40    1101 ATCTAATTAA AATTAATTTT ATCATCATTA TATATTGAGT ACCAGTGTAT
     1151 TTTATATTAC ATATTGATTA CTTTGTTTTT ATTTTGTTTA TATCATTTTA
     1201 CGTTTGTACT ATAAATTATT TCTACAAACA CAAAAACCG ATGCATACGC
     1251 ATCGGCTCAT TTGTAATACA GTATTTATTT ATCTAATCCC ATTTTATCTT
     1301 GAACCACATC AGCTATTTGT TGTGCAAATC TTTCAGCATC TTCATCAGTT
45    1351 GCTGCTTCAA CCATGACACG AACTAATGGT TCTGTTCCAG AAGGTCTTAC
     1401 TAAAAATTCGA CCTTCTCCAT TCATTTCTAC TTCTACTTTA GTCATAACTT
     1451 CTTTAACGTC AACATTTTCT TCAACACGAT ATTTATCTGT TACGCGTACG
     1501 TTAATTAATG ATTGTGGATA TTTTTCATT TGTCCAGCTA ATTCACTTAG

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1551 TGATTTACCA GTCATTTTTA TTACAGAAGC TAATTGAATA CCAGTTAATA
 1601 AACCATCACC AGTTGTATTG TAATCCAYCA TAACGATATG TCCARATKGT
 1651 TCTCCACCTA AGTTATAATT ACCGCGAMGC ATTTCTTCTA CTACATATCT
 1701 GTCGCCAACT TTAGTTTTAT TAGATTTAAT TCCTTCTTGT TCAAGCGCTT
 5 1751 TGAAAAAACC TAAATTACTC ATAACAGTAG AAAACGAATC ATGTCATTAT
 1801 TCAATTCTTG ATTTTTATGC ATTTCTTGAC CAATAATAAA CATAATTTGG
 1851 TCACCGTCAA CGATTTGACC ATTCCTCATCT ACTGCTATGA TTCTGTCTCC
 1901 ATCGCCGTCA AATGCTAACC CAAAATCACT TTCAGTTTCA ACTACTTTTT
 1951 CAGCTAATTT TCAGGATGTG TAAAGCCACA TTTCTCATTG ATATTATATC
 10 2001 CATCAGGGAC TACATCCA

SEQ ID NO. 17

pMP48.reverse Length: 2573 nt

15 1 ATTCGAGCTC GGTACCCGKG GATCCTSYAG AGTCGATCCG CTTGAAACGC
 51 CAGGCACTGG TACTAGAGTT TTGGGTGGTC TTAGTTATAG AGAAAGCCAT
 101 TTTGCATTGG AATTACTGCA TCAATCACAT TTAATTTCTT CAATGGATTT
 151 AGTTGAAGTA AATCCATTGA TTGACAGTAA TAATCATACT GCTGAACAAG
 201 CGGTTTCATT AGTTGGAACA TTTTTTGGTG AAACTTTATT ATAAATAAAT
 20 251 GATTGTAGT GTATAAAGTA TATTTTGCTT TTTGCACTAC TTTTTTTAAT
 301 TCACTAAAAT GATTAAGAGT AGTTATAATC TTTAAAATAA TTTTTTTCTA
 351 TTTAAATATA TGTTTCGTATG ACAGTGATGT AAATGATTGG TATAATGGGT
 401 ATTATGGAAA AATATTACCC GGAGGAGATG TTATGGATTT TTCCAACCTT
 451 TTTCAAAACC TCAGTACGTT AAAAATTGTA ACGAGTATCC TTGATTTACT
 25 501 GATAGTTTGG TATGTACTTT ATCTTCTCAT CACGGTCTTT AAGGGAACATA
 551 AAGCGATACA ATTACTTAAA GGGATATTAG TAATTGTTAT TGGTCAGCAG
 601 ATAATTWTGA TATTGAACTT GACTGCMACA TCTAAATTAT YCRAWWYCGT
 651 TATTCMATGG GGGGTATTAG CTTTAANAGT AATATTCCAA CCAGAAATTA
 701 GACGTGCGTT AGAACAACCTT GGTANAGGTA GCTTTTAAA ACGCNATACT
 30 751 TCTAATACGT ATAGTAAAGA TGAAGAGAAA TTGATTCAAT CGGTTTCAAA
 801 GGCTGTGCAA TATATGGCTA AAAGACGTAT AGGTGCATTA ATTGTCTTTG
 851 AAAAAGAAAC AGGTCTTCAA GATTATATTG AAACAGGTAT TGCCAATGGA
 901 TTCAAAATAT TCGCAAGAAC TTTTAATTAA TGCTTTTATA CCTAACACAC
 951 CTTTACATGA TGGTGCAAKG ATTATTCAAG GCACGAARAT TGCAGCAGCA
 35 1001 GCAAGTTATT TGCCATTGTC TGRWAGTCCT AAGATATCTA AAAGTTGGGT
 1051 ACAAGACATA GAGCTGCGGT TGGTATTTCA GAAGTTATCT GATGCATTTA
 1101 CCGTTATTGT ATCTGAAGAA ACTGGTGATA TTTCGGTAAC ATTTGATGGA
 1151 AAATTACGAC GAGACATTTT AAACCGAAAT TTTTGAAGAA TTGCTTGCTG
 1201 AACATTGGTT TGGCACACGC TTTCAAAGA AAGKKKTGAA ATAATATGCT
 40 1251 AGAAAKTAAA TGGGGCTTGA GATTTATTGC CTTTCTTTT GGCATTGTTT
 1301 TTCTTTTAT CTGTAAACAA TGTTTTTGGA AATATTCTTT AAACACTGGT
 1351 AATTCTTGGT CAAAAGTCTA GTAAAACGGA TTCAAGATGT ACCCGTTGAA
 1401 ATTCCTTTATA ACAACTAAAG ATTTGCATTT AACAAAAGCG CCTGAAACAG
 1451 TTAATGTGAC TATTTTCAGGA CCACAATCAA AGATAATAAA AATTGAAAAT
 45 1501 CCAGAAGATT TAAGAGTAGT GATTGATTTA TCAAATGCTA AAGCTGGAAA
 1551 ATATCAAGAA GAAGTATCAA GTTAAAGGGT TAGCTGATGA CATTCAATTAT
 1601 TCTGTAAAC CTAAATTAGC AAATATTACG CTGAAAAACA AAGTAACTAA
 1651 AAAGATGACA GTTCAACCTG ATGTAAGTCA GAGTGATATT GATCCACTTT
 1701 ATAAAAATTAC AAAGCAAGAA GTTTCACCAC AAACAGTTAA AGTACAGGT
 50 1751 GGAGAAGAAC AATTGAATGA TATCGCTTAT TTAAAAGCCA CTTTTAAAC
 1801 TAATAAAAAG ATTAATGGTG ACACAAAAGA TGTCGCAGAA GTAACGGCTT

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1851 TTGATAAAAA ACTGAATAAA TTAAATGTAT CGATTCAACC TAATGAAGTG
1901 AATTTACAAG TTAAAGTAGA GCCTTTTAGC AAAAAGGTTA AAGTAAATGT
1951 TAAACAGAAA GGTAGTTTRS CAGATGATAA AGAGTTAAGT TCGATTGATT
2001 TAGAAGATAA AGAAATTGAA TCTTCGGTAG TCGAGATGAC TTMCAAATA
5 2051 TAAGCGAAGT TGATGCAGAA GTAGATTTAG ATGGTATTTC AGAATCAACT
2101 GAAAAGACTG TAAAAATCAA TTTACCAGAA CATGTCAC TAAGCACAACC
2151 AAGTGAAACG AAGGCTTATA TAAATGTAAA ATAAATAGCT AAATTAAAGG
2201 AGAGTAAACA ATGGGAAAAT ATTTTGGTAC AGACGGAGTA AGAGGTGTCC
2251 CAAACCAAGA ACTAACACCT GAATTGGCAT TTAAATTAGG AAGATACGGT
10 2301 GGCTATGTTC TAGCACATAA TAAAGGTGAA AAACACCCAC GTGTACTTGT
2351 AGGTCGCGAT ACTAGAGTTT CAGGTGAAAT GTTAGAATCA GCATTAATAG
2401 CTGGTTTGAT TTCAATTGGT GCAGAAGTGA TGCGATTAGG TATTATTTC
2451 ACACCAGGTG TTGCATATTT AACACGCGAT ATGGGTGCAG AGTTAGGTGT
2501 AATGATTTCA GCCTCTCATA ATCCAGTTGC AGATAATGGT ATTAAATTCT
15 2551 TTGSCTCGAC CNCCNNGCTN GCA

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Mutant: NT19

20 **Phenotype:** temperature sensitivity

Sequence map: Mutant NT19 is complemented by pMP49, which contains a 1.9 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 31. Database searches at both the nucleic acid and peptide levels reveal strong similarity at the nucleic acid level to the *rnpA* gene, which encodes the catalytic RNA component RNase P, from the bacilli *B. megaterium*, *B. subtilis*, and *B. stearothermophilus* as well as from other prokaryotes. The strongest similarity observed is to the *rnpA* Genbank entry from *B. subtilis* (Genbank Accession No.M13175; published in Reich, C.. et al. *J. Biol. Chem.*, 261 (1986) 7888-7893).

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP49, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

40

clone pMP49
SEQ ID NO. 18

45

pMP49 Length: 1962 nt

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      1 GTGCTTCCAC CAATACGTTT CACCATATGG AGGATTTCCA ATTAACGCCA
    51 CCGGTTCTTC TGTATCAATT GTTAATGTAT TGACATCTTT TACACTAAAT
   101 TTAATAATAT CAGACAACCC AACTTCTTCA GCGTTACGCT TAGCAATCTC
   151 TACCATTCTT GGATCGATAT CAGAAGCATA TACTTCGATT TCTTTATCAT
  201 AATCAGCCAT CTTATCCGCT TCATCACGGT AATCATCATA AATATTTGCT
  251 GGCATGATGT TCCATTGCTC TGATACGAAC TCGCGATTAA AACCAGGTGC
  301 GATATTTTGA GCAATTAAAC AAGCTTCTAT AGCTATTGTA CCCGAACCGC
  351 AAAATGGATC AATTAAAGGT GTATCACCTT TCCAGTTTGC AAGACGGATT
  401 AAACCTGCTG CCAACGTTTC TTTAATTGGT GCTTCACCTT GTGCTAATCT
  451 ATAACCACGT CTGTTCAAAC CAGAACCTGA TGTGTCGATA GTCAATAATA
  501 CATTATCTTT TAAAATGGCA ACTTCAACAG GGTATTTGGC ACCTGATTCA
  551 TTTAACCAAC CTTTTTCGTT ATATGCGCGA CGTAATCGTT CAACAATAGC
  601 TTTCTTAGTT ATCGCCTGAC AATCTGGCAC ACTATGTAGT GTTGATTAA
  651 CGCTTCTACC TTGAACTGGG AAGTTACCCT CTTTATCAAT TATAGATTCC
  701 CAAGGGAGCG CTTTGGTTTG TTCGAATAAT TCGTCAAACG TTGTGCGTW
  751 AAAACGTCCA ACAACAATTT TGATTGCGTC TGCTGTGCGC AACCATAAAT
  801 TTGCCTTTAC AATTGCACTT GCGTCTCCTT CAAAAAATAT ACGACCATT
  851 TCAACATTTG TTTCATAGCC TAATTCTTGA ATTTCCCTAG CAACAACAGC
  901 TTCTAATCCC ATCGGACAAA CTGCAAGTAA TTGAAACATA TATGATTCTC
  951 CTTTTATACA GGTATTTTAT TCTTAGCTTG TGTTTTTTAT ACATTTCCAA
 1001 CAAATTTAAT CGCTGATACA TTAACGCATC CGCTTACTAT TTTAAACAA
 1051 GGCAGTGTC AATTATCAAG ACAAGGCGTT AATTTTAAGT GTCTTCTT
 1101 CATGAAAAAA GCTCTCCMTC ATCTAGGAGA GCTAAACTAG TAGTGATATT
 1151 TCTATAAGCC ATGTTCTGTT CCATCGTACT CATCACGTGC ACTAGTCACA
 1201 CTGGTACTCA GGTGATAACC ATCTGTCTAC ACCACTTCAT TTCGCGAAGT
 1251 GTGTYTCGTT TATACGTTGA ATTCCGTTAA ACAAGTGCTC CTACCAAATT
 1301 TGGATTGCTC AACTCGAGGG GTTTACCGCG TTCCACCTTT TATATTTCTA
 1351 TAAAAGCTAA CGTCACTGTG GCACTTTCAA ATTACTCTAT CCATATCGAA
 1401 AGACTTAGGA TATTTCAATT CCGTCAAATT AATGCCTTGA TTTATTGTTT
 1451 CAYCAAGCRC GAACACTACA ATCATCTCAG ACTGTGTGAG CATGGACTTT
 1501 CCTCTATATA ATATAGCGAT TACCCAAAAT ATCACTTTTA AAATTATAAC
 1551 ATAGTCATTA TTAGTAAGAC AGTTAACTT TTGTATTTAG TAATTATTTA
 1601 CCAAATACAG CTTTTTCTAA GTTTGAAATA CGTTTTAAAA TATCTACATT
 1651 ATTTGAAGAT GTATTTGTTG TTGTATTATT CGAAGAAAAA CTTTTATTGT
 1701 CCTGAGGTCT TGATGTTGCT ACACGTAGTC TTAATTCCTC TAATTCCTTT
 1751 TTAAGTTTAT GATTCTCTTC TGATAATTTT ACAAATTCAT TATTCATATC
 1801 GGCCATTTTT TGATAATCAG CAATAATGTC ATCTAAAAAT GCATCTACTT
 1851 CTTCTCTTCT ATAGCCACGA GCCATCGTTT TTTCAAAATC TTTTTCATAA
 1901 ATATCTTTTG CTGATAATTT CAATGAAACA TCTGACATTT TTTCCACCTC
 1951 ATTAGAACT TT

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Mutant: NT23

45 Phenotype: temperature sensitivity

Sequence map: Mutant NT23 is complemented by pMP55, which contains a 5.2 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 32. Database searches at both the nucleic acid and peptide levels reveal

limited similarity at the protein level only to *S. aureus* proteins FemA and FemB, suggesting that clone pMP55 contains a new Fem-like protein. Since the Fem proteins are involved in peptidoglycan formation, this new Fem-like protein is likely to make an attractive candidate for screening antibacterial agents. Since clone pMP55 does not map to the same location as the *femAB* locus (data not shown here), the protein is neither FemA nor FemB and represents a novel gene.

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP55, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP55, a 5000 bp genomic fragment

SEQ ID NO. 19

pMP55 Length: 5253 nt

```

1  TAACTGGACT ACWACCGCCA ACTRAGTATT GAATTGTTTT AACATGCTTT
51  TCCTGTTTTA AATATTTTTA AACATCTTTC GCATGATTCA AACTGCTTG
101 CTCCGTTTCA CCAGGCTTCG GTGTATAAGT AATAGCTAAA AATTTATCGT
151 CACCTGCTGA AATAAAGCTA GTGCCTAGTC TCGGTCCTCC AAATACAATA
201 GTTGCAACCA AAATTAATGT ACTTAATATA ATTWCAATCC ACTTATGATT
251 TAATGACCAA TGTAATACTT TTTTATAAGT TGTACTAACA ACACCTAATC
301 CTTCTTGATG TTGTTTATTA CGACGTTTAA CGCCTTTTTT AAATAGTGTA
351 GCTGCCAACG CTGGAACGAG TGTAATTGAC ACTAATAACG ATGCTAATAA
401 ACTAAATGCA ATAGCCAATG CAAAAGGTCT AAACATTTTC CCTACTGAAC
451 CTGATACAAA CACAAGTGGT AAGAAGACGA TAATAGKAAC TAGTGTTCGAT
501 GRCATTATTG GTTTAAATAC TTCAGTTGTC GCACTGATAA TTAAATTTTC
551 ACCTTTTAGT TGGTTCTTCT GAATCTGTTA AGCGTCGATA AATATTTTCA
601 MCAACTACAA TCGAATCGTC TATCACACGT CCAATCGCTA CTGTTAATGC
651 ACCTAACGTT AGTATATTCA ATGAMACATC ACTCAATTTT AGAGCAATAA
701 GCGSCATAAG AAGTGATAAC GGMATCGATA TMATAGAAAT TGCCGTCGTA
751 CGAATGTTTC TTA AAAACAG CAAAATAACT ATAATTGCCA CGRATTGTAC
801 CTAATGATGC TTTTCAACC ATCGTATAAA GTGATTCTC AACAGGCTTT
851 GCAGTATCCA TTGTTTTTGT GACATTAAAA TCTTTATTTT CATCAACGAA
901 TGTATCAATT TTACGTTGTA CATCTTTGGC TACTTGAAC GTATTGGCAT
951 CTTGAGCTTT AGTTATTTGT AGATTAACCG CATCCTTTCC ATTCGTTTTA
1001 GAAATAGAAG TACGCACATC ACCAACTGTA ATATCAGCTA AATCTCCTAG
1051 TTTGCTGTGC GGCATACCAC TTATATTATT TGGTGCTGAC GCTTTTGAAT
1101 TTTGCTGTGG TGATGCCTGA TTAACGTCTG ACATGGCTGA AATTTTGTTT
1151 ATTGTCACCT TGGGATTGAG ATTGCCCTTG TCCTCCTGCC AACGTTAATG

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	1201	GAATATTTAT	GTTTTTAAAA	GCATCAACAG	ATTGATATTG	ACCATCAACA
	1251	ACAATTGATT	TATCTTTATC	ACCAAATTGG	AACAATCCAA	GTGGCGTTGT
	1301	TCTTGTTGCC	GTTTTTAGAT	AGTTTTCTAC	ATCATCAGCA	GTCAACCCAT
	1351	ATTTTCAAGT	TCATTTTGCT	TAAATTTAAG	GGTGATTTC	CGGTTTCGTCT
5	1401	GCCCCATTAA	TTGCGCATTT	TGNACACCAT	CTACCGTTTG	CAATTTTGGT
	1451	ATNAATTGTT	CATTTCAGTAC	TTTCGTTACT	TTTTTCAAGT	CATTCNCTTT
	1501	ATTTGAAAAAT	GAATATGCTA	AAACCGGAAA	AGCATCCATC	GAATTACGTC
	1551	NTANTTCTGG	TTGACCAACT	TCATCTTTAA	ATTTAATTTT	NTNTATTTCT
10	1601	NTNTAAGCT	GTTCTTCTGC	TTTATCCAAA	TCTGTATMT	TTTCATATTC
	1651	AACTGTTACA	ATTGAAGCAT	TTTGTATGGA	TTGCGTTTTA	ACATTTTTC
	1701	CATATGCCAA	TGATCTTACY	TGAWTGTCAA	TTTTACTACT	TATTTTCATCT
	1751	TGGGTACTTT	GTGGCGTTGC	ACCCGGCATT	GTTGTTGTAA	CTGAAATAAC
	1801	TGGATKTTGT	ACATTTGGTA	KTAATTCTMA	TTTCAATTTA	GCACTCGCAT
	1851	ATACACCGCC	CAAGACAACT	WAAACAACCA	TTAMAAAGAT	AGCAAACYTA
15	1901	TTCCCTAAAA	RGAAAATTGT	AATAGCTTTT	TTAWCAACAG	TMCTYCCCCC
	1951	TCTTTCACTA	WAATTCAAAA	AATTATTTTA	CTCAACCATY	CTAWWWTGTG
	2001	TAAAAAAAAT	CTGAACGCAA	ATGACAGYCT	TATGAGCGTT	CAGATTTTCAG
	2051	YCGTTAATCT	ATTTYCGTTT	TAATTTACGA	GATATTTTAA	TTTTAGCTTT
20	2101	TGTTAAACGC	GGTTTAACTT	GCTCAATTAA	TTGGYACAAT	GGCTGATTCA
	2151	ATACATAATC	AAATTCACCA	ATCTTTTCAC	TTAAGTATGT	TCCCCACACT
	2201	TTTTTTAAATG	CCCATAATCC	ATAATGTTCT	GAGTCTTTAT	CTGGATCATT
	2251	ATCTGTACCA	CCGAAATCGT	AAGTTGTTGC	ACCATGTTCA	CGTGCATACT
	2301	TCATCATCGT	ATACTGCATA	TGATGATTTG	GTAAAAAATC	TCTAAATTCA
	2351	TTAGAAGACG	CACCATATAA	GTAATATGAT	TTTGAGCCAG	CAAACATTAA
25	2401	TAGTGCACCA	GAAAGATAAA	TACCTTCAGG	ATGTTCCCTT	TCTAAAGCTT
	2451	CTAGGTCTCG	TTTTAAATCT	TCATTTTTAG	CAATTTTATT	TTGCGCATCA
	2501	TTAATCATAT	TTTGCGCTTT	TTTAGCTTGC	TTTTTCAGATG	TTTTTCATCTT
	2551	CTGCTGCCAT	TTAGCAATTT	CGGCATGAAG	TTCAATTCAAT	TCTTGATTTA
	2601	CTTTTCGCTAT	ATTTTCTTTT	GGATCCAACT	TTACTAAAAA	TAGTTCAGCA
30	2651	TCTCCATCTT	CATGCAACGC	ATCATAAATA	TTTTCAAAGT	AACTAATATC
	2701	ACGCGTTAAG	AAGCCATCGC	GTTCCCCAGT	GATTTTCATT	AACTCAGCAA
	2751	ATGTTTTTAA	ACCTTCTCTA	TCAGATCGTT	CTACTGTCGT	ACCTCGCTTT
	2801	AAAGCCAAGC	GCACTTTTGA	ACGATTTTCGG	CGTTCAAAAC	TATTTAATAA
	2851	CTCATCATCA	TTTTTATCAA	TTGGTGTAAT	CATAGTCATA	CGTGGTTGGA
35	2901	TGTAGTCTTT	TGATAAACCT	TCTTTAAATC	CTTTATGTTT	AAAACCAAGC
	2951	GCTTTCAAAT	TTTGCAAAGC	ATCTGTRCCT	TTATCAACTT	CAACATCAGG
	3001	ATCGRTTTTA	ATTGCATACG	CTTTCTCAGC	TTTAGCAATT	TCTTTTGCAC
	3051	TGTCTAACMA	TGSMTTTAAC	GYTTCCTTAT	TACTATTAAT	CAACAACCAA
40	3101	AACCMCGCGR	RAWTATWACM	TAGSGTATAA	GGTAATTTAG	GTACTTTTTT
	3151	AAAAAGTAAC	TGCGCAACAC	CCTGGAACCT	SMCCGTCACG	ACCTACAGCG
	3201	ATTCTTCGCG	CGTACCATCC	AGTTAATTTT	TTTGTTTCTG	CCCATTTCGT
	3251	TAATTGTAAT	AAATCTCCAT	TTGGGTGGGR	WTTWACAAAT	GCGTCATGTT
	3301	CCTGATTAGG	KGATATGCAT	CTTTTCCATG	ATTTATGATA	TCTCCTTCTA
	3351	TTTAACAATA	CCTTTAATTA	TACAGTTTGT	ATCTTATAGT	GTCGATTTCAG
45	3401	AGCTTGTAAT	AGATTTGAAC	TCTTATTTTT	GGAAATGTCC	ATGCTCCAAT
	3451	TAATAGTTTA	GCAAGTTCAA	ATTTACCCAT	TTTAATTGTG	AATCATTTTA
	3501	TATCTATGTT	TCGTGTTAAA	TTTAATGTTA	TCGTACARTT	AATACTTTTC
	3551	AACTAGTTAC	CTATACTTCA	ATATACTTTC	ATCATCTAAC	ACGATATTCA
	3601	TTTCTAARAA	TGAACCAACT	TGACTTCAAT	GAATAAATTT	TTCCTCAAGC
50	3651	AACCACATTA	ATGTTTCATAT	ACAATTACCC	CTGTTATAAT	GTCAATAATC
	3701	TAACAATGAG	GTGTTTGATA	TGAGAACAAT	TATTTTAAGT	CTATTTATAA

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5  3751 TTATGRACAT CGTTGCAATC ATTATGACAT TGAGTCAACC TCTCCACCGT
    3801 GAATTACTTT AGTTTACGGG TTATACTTAT CTTTTTCACA TTTATATTAT
    3851 CAATCTTTTT CATTTTAATT AAGTCATCAC GATTAAATAA TATATTAACG
    3901 ATTMWWTCCA TTGTGCTTGT CATTATTCAT ATGGGCATTG TCGCTCATAG
    3951 CACTTACGTA TATTTATACT AATGGTTCAA AGCGATAAAT AGCACCTCTG
    4001 ATAAAAATTG AATATGGTGA AGTTGCTTGT GCGTCTTTTA TGATAACCGA
    4051 ATGATATTTT GAAACTTTAC CATCTTCAAT TCTAAAATAA ATATCATCAT
    4101 TTTTTAAAAAT CAAATCTGTG TAATGGTCAT TTYKTCHACA ATGTCCATAT
    4151 CAARCCATTT CAACCAATTC GATACTGTWK GTGATCGGTT TTTACTTTTC
10  4201 ACAATAACAG TTTCAAWTGA AAATTGTTTT TGAAAATATT TTTGCAATTT
    4251 TTTAGTACGC ATGGAATCAC TTTCTTCCCA TTGAATAAAA AATGGTGGCT
    4301 TAATTTTCATC ATCATCCTGA TTCATTATAT AAAGCAATTG CCACTTTACC
    4351 TWCACCATCT TTATGTGTAT CTCTTTCCAT TTGAATCGGC CCTACTTCTT
    4401 CAACCTGCTC ACTNIGTAGT TTATTTTTTA CTGCCTCTAT ATCATTTGTA
15  4451 CGCAAAACAAA TATTTATTAA AGCCTTGCTC ATACTTCTCT TGAACAATTT
    4501 GAGTAGCAAA AGCGACTCCG CCTTCTATCG TTTTGTCCAT CTTTTTCAAC
    4551 TTTTCATTAT TTTACTACAT CTAGTAGCTC AAGATAATTT CATTGATATW
    4601 ACCTAAKKTa TTGAATGTTC CATATTTATG ATGATACCCA CCTGAATGTA
    4651 ATTTTATAAC ATCCTCCTGG AAAACTAAAC CGATCTAACT GATCTATATA
20  4701 ATGAATGATG TGATCANATT TCAATATCAT TAGTATCCCC CTATTTACAT
    4751 GTAATTACGC TTATTTTTAAA CAAAGTAWAA TTATTTTTGC YCTTAATAAT
    4801 TATATAKTGA YYYCWAATTG CTCCCGTTTT ATAATTACTA TTGTTGTAAA
    4851 ARGGTTAGCT AAGCTAACTA TTTTGCCTTA GGAGATGTCA CTATGCTATC
    4901 ACAAGAATTT TTCAATAGTT TTATAACAAT ATAYCGCCCC TATTTAAAAT
25  4951 TAGCCGAGCC GATTTTtagra AAACACAATA TATATTATGG CCAATGGTTA
    5001 ATCTTACGCG ATATCGCTAA ACATCAGCCC ACTACTCTCA TTGNAATTTT
    5051 ACATAGACGG GCAATTGAAA AGCCTACTGC AAGAAAAAAT TTAAAAGCTC
    5101 TAATAGGAAA TGACCTTATW ACAGTAGAAA ACAGNTTAGA GGATAAACNA
    5151 CAAAAGNTTT TAACTTTAAc ACCTAAAGGG CATKAATTAT ATGAGATTGT
30  5201 TTGTCTTGAT GNACAAAAGC TCCNACAAGC AGNNAGTTGC CAAAACAAAG
    5251 ATT

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35 Mutant: NT27

Phenotype: temperature sensitivity

Sequence map: Mutant NT27 is complemented by pMP59, which contains a 3.2 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 33. Database searches at both the nucleic acid and peptide levels reveal strong peptide-level similarities to two hypothetical ORFs from *B. subtilis*. These hypothetical ORFs are also found in other bacteria, but in all cases, nothing has been reported in the literature about the functions of the corresponding gene products.

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP59, starting with the

standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP59

SEQ ID NO. 20

pMP59 Length: 3263 nt

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1  ACATTGAMAA AGATCACCCA TTACAACCAC ATACAGATGC AGTAGAAGTT
51  TAAACACAT TTTTCTAATT ATCAAAGCTT AGGATAAATA TGATGTCCTA
101 AGCTTTTCCT TTTACAACCTT TTTCGAATAA ACAACAGTTA AATATATTCA
151 CCTTTCTACC AAACTTTTTTA TCCCCTCATT TAAATTTTAC CGGKYTCATA
201 TAAATCCTT TAATTCTTTC TTAACATTAW TTTWTWATCT CTACATYTAT
251 TTTAATAAAT AGAACTGCAC ATTTATTTCGA AATACTTAGA TTTCTAGTGA
301 GATAAACTGC TTTATTTATT ATCATTCATC ATGTAAAATA AGATTTAACT
351 GAAATTTTAG TGTTATTTCA CTAATTTTTT AAAATGAACG ACATGATGAA
401 CCTAGTTATT AACCAAATCG TTATTAAGTT ACATTATAGA GATGATTGGA
451 ATGAATTTAT CGATATATAC TCCAATACGA TTTTACTAGG GTTAACAATA
501 AATTAAACAA ACATTCTTAG GAGGRATTTT TAACATGGCA GTATTTAAAG
551 TTTTTTATCA ACATAACAGA GTACGAGGTR RTTGTGCGTG AAAATACACA
601 ATCACTTTAT GTTGAAGCTC ARACAGAAGA ACAAGTAGCG TCGTTACTTG
651 AAAGATCGTA ATTTTAATAT CGAATTTATC ACTAAATTAG AGGGCGCACA
701 TTTAGATTAC GAAAAAGAAA ACTCAGCAAC ACTTTAATGT GGAGATTGCT
751 AAATAATGAA ACAATTACAT CCAAATGAAG TAGGTGTATA TGCACCTGGA
801 GGTCTAGGTG AAATCGGTAA AAATACTTAT GCAGTTGAGT ATAAAGACGA
851 AATTGTCATT ATCGATGCCG GTATCAAATT CCCTGATGAT AACTTATTAG
901 GGATTGATTA TGTTATACCT GACTACACAT ATCTAGTTCA AAACCAAGAT
951 AAAATTGTTG GCCTATTTAT AACACATGGT CACGAAGACC ATATAGGCGG
1001 TGTGCCCTTC CTATTAATAAC AACTTAATAT ACCTATTTAT GGTGGTCCCT
1051 TAGCATTAGG TTTAATCCGT AATAAACTTG AAGAAACATC ATTTATTACG
1101 TACTGCTAAA CTAAATGAAA TCAATGAGGA CAGTGTGATT AAATCTAAGC
1151 ACTTTACGAT TTCTTTCTAC TTAACTACAC ATAGTATTC TGAACCTTAT
1201 GGCGTCATCG TAGATACACC TGAAGGAAAA KTAGTTCATA CCGGTGACTT
1251 TAAATTTGAT TTTACACCTG TAGGCAAACC AGCAAACATT GCTAAAATGG
1301 CTCAATTAGG CGAAGAAGGC GTTCTATGTT TACTTTTCAGA CTCAACAAAT
1351 TCACTTGTGC CTGATTTTAC TTTAAGCGAA CGTTGAAGTT GGTCAAAACG
1401 TTAGATAAGA TCTTCCGTAA TTGTAAAGGT CCGTATTATA TTTGCTACCT
1451 TCGCTTCTAA TATTTACCGA GTTCAACAAG CAGTTGAAGC TGCTATCAAA
1501 AATAACCGTA AAATTGTTAC KTTCCGTCCG TTCGATGGAA AACAATATTA
1551 AAATAGKTAT GGAACCTGGT TATATTAAAG CACCACCTGA AACATTTATT
1601 GAACCTAATA AAATTAATAC CGTACCGAAG CATGAGTTAT TGATACTATG
1651 TACTGGTTCA CAAGGTGAAC CAATGGCAGC ATTATCTAGA ATTGCTAATG
1701 GTACTCATAA GCAAATTAAA ATTATACCTG AAGATACCGT TGTATTTAGT
1751 TCATCACCTA TCCCAGGTAA TACAAAAAGT TATTAACAGA ACTATTAATT
1801 CCTTGATAAA AGCTGGTGCA GATGTTATCC ATAGCAAGAT TTCTAACATC
1851 CATACTCAG GGCATGGTTC TCAAGGGTGA TCAACAATTA ATGCTTCCGA
1901 TTAATCAAGC CGAAATATTT CTTACCTATT CATGGTGAAT ACCGTATGTT
1951 AAAAGCACAT GGTGAGACTG GTGTTGAATG CGSSKTTGAA GAAGATAATG

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2001 TCTTCATCTT TGATATTGGA GATGTCTTAG CTTTAACACM CGATTTCAGCA
 2051 CGTAAAGCTG KTCGCATTCC ATCTGGTAAT GWACTTGTTG ATGGTAGTGG
 2101 TATCGGTGAT ATCGGTAATG TTGTAATAAG AGACCGTAAG CTATTATCTG
 2151 AAGAAGGTTT AGTTATCGTT GTTGTTAGTA TTGATTTTAA TACAAATAAA
 5 2201 TTAATTTCTG GTCCAGACAT TATTTCTCGA GGATTTGTAT ATATGAGGGA
 2251 ATCAGGTCAA TTAATTTATG ATGCACAACG CMAAAWCAA ACTGATGTTT
 2301 ATTAGTWAGT TWAATCCAAA ATAAAGAWAT TCAATGGCAT CAGATTAAAT
 2351 CTTCTATCAT TGAAACATTA CAACCTTATT TATTKGAAAA AACAGCTAGR
 2401 AAACCAATGA TTTTACCAGT CATTATGGAA GGTAACGAA CAAAARGAAT
 10 2451 CAAACAATAA ATAATCAAAA AGCTACTAAC TTTGAAGTGA AGTTTAAATT
 2501 AAACCTACCC ACCCATTTGTT AGTAGCTTTT TCTTTATATA TGATGAGCTT
 2551 GAGACATAAA TCAATGTTCA ATGCTCTACA AAGTTATATT GGCAGTAGTT
 2601 GACTGAACGA AAATGCGCTT GTWACAWGCT TTTTTCATT STASTCAGGG
 2651 GCCCCWACAT AGAGAATTTT GAAAAGAAAT TCTACAGGCA ATGCGAGTTG
 15 2701 GGGTGTGGGC CCCAACAAAG AGAAATTGGA TTCCCCAATT TCTACAGACA
 2751 ATGTAAGTTG GGGTGGGACG ACGGAAATAA ATTTTGAGAA AATATCATT
 2801 CTGTCCCCAC TCCCGATTAT CTCGTCGCAA TATTTTTC AAAGCGATT
 2851 AAATCATTAT CCATGTCCCA ATCATGATTA AATATCACC TATTTCTAAA
 2901 TTAATATTTG GATTTGGTGA AATGATGAAC TCTTTGCCTC GTTTAATTGC
 20 2951 AATAATGTTA ATTCCATATT GTGCTCTTAT ATCTAAATCA ATGATAGACT
 3001 GCCCCGCCAT CTTTTCAGTT GCTTTCAATT CTACAATAGA ATGCTCGTCT
 3051 GCCAACTCAA GATAATCAAG TACACTTGCA CTCGCAACAT TATGCGCNAT
 3101 ACGTCTACCC ATATCACGCT CAGGGTGCAC AACCGTATCT GCTCCAATTT
 3151 TATTTAAAAT CTTTGCNTGA TAATCATTTT GTGCTCTTAG CAGTTACTTT
 25 3201 TTTTACACCT AACTCTTTTA AAATTAAAGT CGTCAACGTA CTTGNTTGAA
 3251 TATTTTCACC AAT

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Mutant: NT28

Phenotype: temperature sensitivity

Sequence map: Mutant NT28 is complemented by pMP60, which
 contains a 4.7 kb insert of *S. aureus* genomic DNA. A
 35 partial restriction map is depicted Fig. 34, along with
 open boxes to indicate the percentage of the clone for
 which DNA sequence has been obtained.. Database searches
 at both the nucleic acid and peptide levels reveal identity
 of clone pMP60 at both the nucleic acid and peptide levels
 40 to the *polC* gene, encoding DNA Polymerase III alpha
 subunit, from *S. aureus* (Genbank Accession No. Z48003;
 unpublished as of 1995). The relative size and orientation
 of the complete ORF encoding Pol III is depicted by an
 arrow in the map.

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DNA sequence data: The following DNA sequence data was
 generated by using the standard sequencing primers SP6 and

T7, and can be used to demonstrate identity between clone pMP60 and Genbank entry Z48003:

subclone 1022, a 900 bp EcoR I fragment

5 SEQ ID NO. 21

1022.sp6 Length: 510 nt

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1   GGGTACCGAG CTCGAATTCG AGGTGTACGG TAGAAATACT TCACCAATGA
51  TGCACCTACA ATTTTAAATA GATTTTNAAG ACCTTGTTGG TTTTGTACAA
10  101 TTAATGTGAC ATGACTAGGT CTTGCACGTT TATATGCATC TNCATTACTG
151 AGTTTTTTGT TGATTTCGTT ATGATTTAAT ACGCCTAATT CTTTCATTTG
201 TTGAACCATT TTNATGAAAA TGTAAGCTGT TGCTTCTGTA TCATAAATGG
251 CACGGTGATG TTGCGTTAAT TCTACGCCAT ATTTTTTAGC CAAGAAATTC
301 AAACCATGTT TACCATATTC AGTATTAATC GTACGNGATA ATTCTAAAGT
15  351 ATCGNTAACA CCATTCGTTG ATGGTCCAAA CCCAAGACGT TCATATCCCCG
401 TATCGATGNN GCCCATATCA AACGGAGCAT TATGCGTTAC GGTTTTTCGNA
451 TCGGCAACCC TTCTTAAACT CTGTAAGNAC TTCTTCATTT CAGGGGATCT
501 NCTANCATAT

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20 subclone 1023, a 1200 bp EcoR I fragment

SEQ ID NO. 22

1023.sp6 Length: 278 nt

```

1   GGGTACCGAG CTCGAATTCT ACACGCTTTT CTTCAGCCTT ATCTTTTTTTT
25  51 GTCGCTTTTT TAATCTCTTC AATATCAGAC ATCATCATAA CTAAATCTCT
101 AATAAATGTA TCTCCTTCAA TACGNCCTTG AGCCCTAACC CATTTACCAA
151 CANTTAGNGC TTTAAATGT TCTAAATCAT CTTTGTTTTT ACGAGTAAAC
201 ATTTTTTAAA CTAAAGNGTC CGTATAGTCA GTCACCTTAA TTTCTACGGT
251 ATGGNGGCCA CTTTTAAGTT CTTTTAAG

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subclone 1024, a 1400 bp EcoR I fragment

SEQ ID NO. 23

1024.sp6 Length: 400 nt

```

35  1   GGGTACCGAG CTCGAATTCT GGTACCCCAA ATGTACCTGT TTTACATAAA
51  ATTTTCATCT CAGTAACACC CAAACTTTCA GGTGTACTAA ATATCTGCAT
101 AACTNCTTTA TCATCTACAG GTATTGTTTT TGGNTCAATT CCTGATAAAT
151 CTTGAAGCAT ACGAATCATT GTTGGNTCAT CGTGTCCAAG TATATCANGT
201 TTTAATACAT TATCATGAAT AGAATGGAAA TCAAAATGTG TCGTCATCCA
40  251 TGCTGAATTT TGATCATCGG CAGGATATTG TATCGGCGTA AAATCATAAA
301 TATCCATGTA ATCAGGTACT ACAATAATAC CCCCTGGNTG CTGTCCAGTT
351 GTACGTTTAA CACCTGTACA TCCTTTAACG NGTCGATCTA TTTCAGCACC

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subclone 1025, a 1200 bp EcoR I/ Hind III fragment

45 SEQ ID NO. 24

1025.sp6 Length: 528 nt

```

      1 GATCATTTCG ATCCATAGCT TCACTTATTT NTCCAGAAGC TAGCGTACAA
    51 TCATTTAAAT CTACGCCACC TTCTTTATCA ATAGAGATTC TAAGAAAATN
   101 ATCTCTACCC TCTTTGACAT ATTCAACGTC TACAAGTTCA AAATTCAAGT
   151 CTTCCATAAT TGGTTAACA ATCACTTCTA CTTGTCCTGT AATTTNCTC
  5 201 ATACAGGCCT CCCTTTTGG CAAATAGAAA AGAGCGGGAA TCTCCCACTC
   251 TTCTGCCTGA GTTCACTAAT TTTTAAGCAA CTTAATTATA GCATAAGTTT
   301 ATGCTTGAAA CAAATGACTT CACTATTAAT CAGAGATTCT TGTAAGAGTT
   351 TGTCCCTTTA TTCACCATTT ACATTTGAAT NGNCTCGTNA GNCATTGTAA
   401 AGAGATNCGG GCATAATTTT GTGTCCAGCA TCAATTTTGG TATTTCTTGT
 10 451 CTTACGGCTT ACGGTTNATT AAATACCTNG GNTTTTNTC TTTTACCTNT
   501 NATATNTCGN ANGNTGGGNT TTTTCNNG

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15 **Mutant: NT29**

Phenotype: temperature sensitivity

Sequence map: Mutant NT29 is complemented by pMP62, which contains a 5.5 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 35, along with open boxes to indicate the percentage of the clone for which DNA sequence has been obtained.. Database searches at both the nucleic acid and peptide levels reveal identity between clone pMP62 and the *gyrBA* locus of *S. aureus* (Genbank Accession No. M86227; published in Margerrison, E.E., et al. *J. Bacteriology*, 174 (1992) 1596-1603), which encodes DNA gyrase (EC 5.99.1.3). Arrows above the restriction map indicate relative size and position of the ORFs, demonstrating that both *gyrB* and *gyrA* genes are fully contained within clone pMP62 and are likely to be expressed.

DNA sequence data: The following DNA sequence data are those obtained from subclones of clone pMP62, using standard sequencing conditions and the primers T7 or SP6. These data can be used to demonstrate identity between the pMP62 clone and Genbank entry M86227.

subclone 29.2e.a, a 550 bp EcoR I fragment

SEQ ID NO. 25

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29.2e.a.sp6 LENGTH: 557 nt

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      1 CAGCCGACAG TTNACAACCA GCNTCACCGT NAGACAGCAA ACGCCACAAA
    51 CTACAAGGNT CCAATGNCT AGACAATACT GGTGNAAGGC ANGTAATAAT
   101 ACGACATTAA CATTGATGA TCCTGCCATA TCAACAGNTC AGAATAGACA
  45 151 GGATCCAACCT GTAAGTGTTA CAGATAAAGT AAATGGTTAT TCATTAATTA

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201 ACAACGGTAA GATTGGTTTC GTTAACTCAG AATTAAGACG AAGCGATATG
 251 TTTGATAAGA ATAACCCTCA AAACATCAA GCTAAAGGAA ACGTGGCTGC
 301 ATTAGGTCGT GTGAATGCAA ATGATTCTAC AGATCATGGT AACTTTAACG
 351 GTATTTCAAA AACTGTAAAT GTAAAACCAG NTTCAGAATT AATTATTAAC
 5 401 TTTACTACTA TGCAAACCGG ATAGTNAGCA AGGTGCAACA AATTTAGTTA
 451 TTAAAGGATG CTAAGGAANN TACTGNNTTA GCACCTGTAA AATGTTGCTT
 501 AGGCTGGTCC TGCACATTTA TTTTAAGGTC CNNCTTGTNC TGNTNGGCTC
 551 TNGGGGG

10 SEQ ID NO. 26

29.2e.a.t7 LENGTH: 527 nt

1 GTCGATCAGC ATCATTGGTA CTTTAAATAA ATGTGCAGTA CCAGTCTTAG
 51 CAACATTTAC AGTTGCTAAT TCAGTATTTT CNTTAGCATC TTTAATAACT
 101 AANTTTNTNG CACCTTGCNT ACTATTCGTT TGCATAGTAG TAAAGTTAAT
 15 151 AATTAATTCT GANTCTGGTT TTACATTTAC AGTTTTTGAA ATACCGTTAA
 201 AGTTACCATG ANCTGTAGNA TCATTTGCNT TCACACGGCC TAATGCAGCC
 251 NCGGTTCCCT TAGCTTGATA GTTTTGAGGG GTATTCTTAT CAAACATATC
 301 GNTTCGGCTT AATTCTGAGG TAACTGGNAC CNATCTTTAC CNTTGTTAAT
 351 TAATGGNTTC CCCTTTACNT TAATCTGTAA CAGTTACAGT TGGGTCCCCG
 20 401 TCTATTCTCA TCTGTTGGTA TGGCAGGGTC ACCACAATGN TAATGTCGGT
 451 TTATACTGGN NTCNCCCNA TTGCTTAGGT TTGGNGCTTG NGGTGTGCGN
 501 TTNCTNGCTT CAGGGGNCTG CTGGGTT

subclone 29.2h.2a, a 1800 bp Hind III fragment

25 SEQ ID NO. 27

29.2h.2a.sp6 LENGTH: 578 nt

1 TGTGAGCTCC CATNACCACC AGTGCNNCA TTGCCTGGGC TACCGATTGT
 51 CAATTTAAAG TCTTCATCTT TAAAGAAAAT TTCAGTACCA TGTTTTTTAA
 30 101 GTACAACAGT TGCACCTAAA CGATCAACTG CTTACAGATT ACGCTCATAT
 151 GTCTGTTCTT CAATAGGAAT ACCACTTAAT CGTTCCCATT CTTGAGGTG
 201 TGGTGTAAG ATCACACGAC ATGTAGGTAA TTGCGGTTTC AGTTTACTAA
 251 AGATTGTAAT CGCATCGCCG TCTACGATTA AATTTTGATG CGGTTGTATA
 301 TTTGTAGTA GGAATGTAAT GGCATTATTT CCTTTGAAAT CAACGCCAAG
 35 351 ACCTGGACCA ATTAGTATAC TGTCAGTCAT TTCAATCATT TTCGTCAACA
 401 TTTTCGTATC ATTAATATCA ATAACCATCG CTTCTGGGCA ACGAGAATGT
 451 AATGCTGAAT GATTTGTTGG ATGTGTAGTA CAGTGATTAA ACCACTACCG
 501 CTAAATACAC ATGCACCGAG CCGCTAACAT AATGGCACCA CCTAAGTTAG
 551 CAGATCGGCC CTCAGGATGA AGTTGCAT

40

SEQ ID NO. 28

29.2h.2a.t7 LENGTH: 534 nt

1 CGAGCCAGCA GNTTGCAGCG GCGTGTCCCA TAACTAAGGT GGTGCCATTA
 51 TGTNAGCGGC TCGTCCATGT NTATTTGGCG GTAGTGGTTT AATCACTGTA
 45 101 GCTACACATC CAACAAATCA TTCAGCATTA CATTCTCGTN GCCCAGAAGC
 151 GATGGTTATT GATATTAATG ATACGAAAAT NTTGACGAAA ATNATTGAAA
 201 TGA CTGACAG TATACTAATN GGNCCAGGTC TTGGCGTTGA TTTCAAAGGA
 251 AATAATGCCA TTNCATTCCT ACTACAAAAT ATACAACCGC ATCAAAATTT
 301 AANCGTAGAC GGCGNTGCGA TTNCAATCTT TNGTAACTG NAACCGCAAT

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351 TACCTACATG TNGTGTGNNC TTNACACCAC ACCTCAAAGG NNTGGGNCGG
401 TTANGTGGTA TTCCNNTTGN GGACAGGCAT ATGGNGCGTA ATCGTGNAGC
451 AGTTGNTCGT TTAGGNGCAC TNTNGTCCTT AAAAAACATG GTCTGNATNT
501 CCTTTAANGN NGNNGCTTTA AATTGGCAAT CGGT

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5

subclone 29.2he, 2400 bp Hind III, EcoR I fragment
SEQ ID NO. 29

29.2he.1.sp6 LENGTH: 565 nt

```

10      1 ACCATTCAACA GTGNCATGCA TCATTGCACA CCAAATGNTG TTTGAAGAGG
      51 TGTTTGTGTTG TATAAGTTAT TTAAAATGAC ACTAGNCATT TGCATCCTTA
     101 CGCACATCAA TAACGACACG CACACCAGTA CGTAAACTTG TTTCATCACG
     151 TAAATCAGTG ATACCGTCAA TTTTCTTGTC ACGAACGAGC TCTGCAATTT
     201 TTTCAATCAT ACGAGCCTTA TTCACCTGGA AAGGAATTTT AGTGACAACA
15     251 ATACGTTGAC GTCCGCCTCC ACGTTCCTCA ATAACGAC GAGAACGCAT
     301 TTGAATTGAA CCACGNCCTG TTTCATATGC ACGTCTAATA CCACTCTTAC
     351 CTAAATAAAG TCCNGCAGTT GGGGAATCAG GACCTTCAAT ATCCTCCATT
     401 AACTCAGCAA ATTGNAATNT CAAGGGGTCT TTAAGTTAAG GCTNAGNNCA
     451 CCCTTGTTA ATTCTGTAA GTTATTGTGG TGGGATATTT CGGTTGCCAT
20     501 NCCTNCCNCG GGTACCCNNA TGCACCCNTT GGGTAATNAG GNTTGGGGGT
     551 TTGTGCCCCG TAAGC

```

SEQ ID NO. 30

29.2he.1.t7 Length: 558 nt

```

25      1 CGCAAAACGT CACAGAAANG NACTNCCTAA TGCACTAATG AAGGGCGGTA
     51 TTAAATCGTA CGTTGAGTTA TTGANCNAA AATAAAGGAA CCTATTCATG
     101 AATGAGCCAA TTTATATTCA TCAATCTAAA GATGATATTG ANGTAAGAAAT
     151 TGCNATTCAN TATAACTCAG GATATGCCAC AAATCTTTTA ACTTACGCAA
     201 ATAACATTCA TACGTATGAN GGTGGTACGC ATGANGACGG ATTCAAACGT
30     251 GCATTTACGC GTGTCTTAAA TAGTTATGGT TTAAGTAGCA AGATTNTGTA
     2301 AGANGGAAAA GNTAGNCTTT CTGGTGAAGN TACACGTGAA GGTATNNCNG
     351 CNNTTNTATC TNTCAAACNT GGGGNTCCNC AATTNGGAGG TCAAACGGGG
     401 CAAAAATTTG GGNNTTCTGT AGTGCGTCAN GTTGTNGGTN AATTATTCNN
     451 NGNGNCTTTT TACNGTTTTN CTTGNAAAT CCNCNAGTCG GNCGTNCNGT
35     501 GGTTTNAAA AGGGTTTTTT GNGGCACGTG NACGTGTTNT TCGGAAAAAA
     551 AGCGGGTT

```

40 Mutant: NT31

Phenotype: temperature sensitivity

Sequence map: Mutant NT31 is complemented by pMP64, which contains a 1.4 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 36. Database searches at both the nucleic acid and peptide levels reveal strong similarity at the nucleic acid and peptide levels to the *aroE* gene of *B. aphidicola* (Genbank Accession No.

U09230; unpublished as of 1995), which encodes the shikimate-5-dehydrogenase protein (EC 1.1.1.25). Strong similarities also exist at the peptide level to the *aroE* genes from *E. coli* and *P. aeruginosa*. The size and relative position of the predicted AroE ORF within the pMP64 clone is depicted in the restriction map by an arrow.

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP64, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP64
SEQ ID NO. 31

20 pMP64 Length: 1508 nt

```

      1 AGTSGWTCCG TGTGCATAGG TRTGAAC TTT GAACCACCAC GTTTAATTTT
     51 ATCGTCACAA ATATCTCAA AACCAAGCTC GTCGATAATC ATCTGTATCA
    101 TTGTTAATCT GTGCTGAACG TCTATAAAAT CATGGTGCTT TTTCAATGGA
    25 151 GACATAAAAC TAGGTAAAAA ATAAAATTCA TCTGGCTGTA ATTCATGAAA
     201 TACTTCGCTA GCTACTATCA TATGTGCAGT ATGGATAGGG TTAAACTGAC
     251 CGCCGTAAAG TACTATCTTT TTCATTATTA TGGCAATTCA ATTTCTTTAT
     301 TATCTTTAGA TTCTCTATAA ATCACTATCA TAGATCCAAT CACTTGCAC
     351 AATTCATAT GAGTAGCTTC GCTTAATGTT TCAGCTAATT CTTTTTTATC
    30 401 ATCAAAGTTA TTTTGTAGTA CATGTACTTT AATCAATTCT CTGTTTTCTA
     451 ACGTATCATC TATTTGTTTA ATCATATTTT CGTTGATACC GCCTTTTCCA
     501 ATTTGAAAAA TCGGATCAAT ATTGTGTGCT AAACCTCTTA AGTATCTTTT
     551 TTGTTTGCCA GTAAGCATAT GTTATTCTCC TTTTAATTGT TGTAAAACTG
     601 CTGTTTTTAT AGAATTAATA TCAGCATCTT TATTAGTCCA AATTTTAAAG
    35 651 CTTTCCGCAC CCCTGGTAAA CAAACATATC TAAGCCATTA TAAATATGGT
     701 TTCCCTTGCG CTCTGCTTCC TCTAAAATAG GTGTTTTATA CGGTATATAA
     751 ACAATATCAC TCATTAAAGT ATTGGGAGAA AGATGCTTTA AATTAATAAT
     801 ACTTTCGTTA TTTCCAGCCA TACCCGCTGG TGTGTATTA ATAACGATAT
     851 CGAATTCAGC TAAATAACTT TTCAGCATCT GCTAATGAAA TTTGGTTTAT
    40 901 ATTTAAATTC CAAGATTCAA AACGAGCCAT CGTCTATTTC GCAACAGTTA
     951 ATTTGGGCTT TACAAATTTT GCTAATTCAT AAGCAATACC TTTACTTGCA
    1001 CCACCTGCGC CCAAAATTAA AATGTATGCA TTTTCTAAAT CTGGATAAAC
    1051 GCTGTGCAAT CCTTTAACAT AACCAATACC ATCTGTATTA TACCCTATCC
    1101 ACTTGCCATC TTTTATCAAA ACAGTGTTAA CTGCACCTGC ATTAATCGCT
    45 1151 TGTTTCATCA CATAATCTAA ATACGGTATG ATACGTTCTT TATGAGGAAT
    1201 TGTGATATTA AAGCCTTCTA ATTCTTTTTT CGAAATAATT TCTTTAATTA
    1251 AATGAAAATC TTCAATTGGA ATATTTAAAG CTCATAAGT ATCATCTAAT
    1301 CCTAAAGAAT TAAAATTGTC TCTATGCATA ACGGGCGACA AGGAATGTGA

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1351 AATAGGATTT CCTATAACTG CAAATTTTCAT TTTTSTAATC ACCTTATAAA
1401 ATAGAATTTTC TTAATACAAC ATCAACATTT TTAGGAACAC GAACGATTAC
1451 TTTAGCCCCCT GGTCTTATAG TTATAAAGCC TAGACCAGAG ATCGACCTGC
1501 AGGCAGCA

```

5

Mutant: NT33a

Phenotype: temperature sensitivity

10 **Sequence map:** Mutant NT33a is complemented by pMP67, which contains a 1.8 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 37. Database searches at both the nucleic acid and peptide levels reveal strong peptide-level similarities to ORFs of unknown

15 function in *Synechococcus* sp. (identified as "orf2" in Genbank Accession No. L19521), *M. tuberculosis* (Genbank Accession No. U00024) and *E. coli* (Genbank Accession No. M86305).

20 **DNA sequence data:** The following DNA sequence data represents the sequence of clone pMP59, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR

25 primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP67

SEQ ID NO. 32

30 pMP67 Length: 1810 nt

```

1 CGCGTCTTCC AAATTTTCNAA AGCTGTAAAA AGTTATTAAA TCAAATCTTG
51 CGAATTTGGA TTAGAGGCA CAATCTGANG TTTATAAAAN TAATGCAGAT
101 AGAGCTTTAA AAGCNTTGTC AAAACGTGAT ATTCAATTTG ATNTCATTTC
35 151 CTTAGATCCA CCTTATAATA AAGGTCTCAT TGATAAAGCT TTAATACTAA
201 TTTAGAGGTT TAATTTATTG AAAGAAAATG GTATCATCGT TTGTGAATTT
251 AGCAATCATG AAGAAATAGA TTATCAACCG TTTAATATGA TTAAACGTTA
301 CCATTATGGG TTGACAGACA CATTGTTATT AGAAAAGGGA GAATAGCATG
351 GAACATACAA TAGCGGTCAT TCCGGGTAGT TTTGACCCCA TTACTTATGG
40 401 TCATTTAGAC ATTATTGAGA GAAGTACAGA TAGATTTGAT GAAATTCATG
451 TCTGTGTTCT TAAAAATAGT AAAAAAGAAG GTACGTTTAG TTTAGAAGAG
501 CGTATGGATT TAATTGAACA ATCTGTAAA CATTACCTA ATGTCAAGGT
551 TCATCAATTT AGTGGTTTAC TAGTCGATTA TTGTGAACAA GTAGGAGCTA
601 AAACAATCAT ACGTGGTTTA AGAGCAGTCA GTGATTTTGA ATATGAATTA
45 651 CGCTTAACTT CMATGAATAA AAAGTTGAAC AATGAAATTG AAACGTTATA
701 TATGATGTCT AGTACTAATT ATTCATTTAT AAGTTCAAGT ATTGTTAAAG

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5      751 AAGTTGCAGC TTATCGAGCA GATATTTCTG AATTCGTTCC ACCTTATGTT
      801 GAAAAGGCAT TGAAGAAGAA ATTTAAGTAA TAAAAATAAC AGTATTTTAG
      851 GTTTATCATG GTTTACAATC CTAAAATACT GTTTTCATTT GTTAACGATA
      901 TTGCTGTATG ACAGGCGTGT TGAAATCTGT TTGTTGTTGC CCGCTTATTG
10     951 CATTGTATAT GTGTGTTGCT TTGATTTTCAT TTGTGAAGTA ATGTGCATTG
      1001 CTTTTGTTAA TATTGGTTAT ATATTGTCTT TCTGGGAACG CTGTTTTTAA
      1051 ATGCTTTAAA TATTGTCTGC CACGGTCGTT CATCGCTAAT ACTTTAACTG
      1101 CGTGAATGTT ACTCGTAACA TCTGTAGGTT TAATGTTTAA TAATACATTG
      1151 ATTAACAGTC TTTGGATATG CGTATATGTA TAACGCTTTG TTTTATAGTAA
15     1201 TTTTACAAAA TGATGAAAAT CAGTTGCTTC ATAAATGTTA GATTTCAAAC
      1251 GATTTTCAAA ACCTTCAGTA ACAGTATAAA TATTTTTTAA TGAATCTGTA
      1301 GTCATAGCTA TGATTTGATA TTCAAATAT GGAAATATTT GATTTAATGT
      1351 WATATGAGGT GTTACGTACA AGTGTTGAAT ATCTTTAGGT ACCACATGAT
      1401 GCCAATGATC ATCTTGACTA ATGATTGATG TTCTAATAGA TGTACCACTT
15     1451 SCAAACTGAT GGTGTTGAAT TAATGAATCA TGATGTTGAG CATTTTCTCG
      1501 TTTGATAGAA ATTGCATTGA TGTTTTTAGC ATTTTTAGCA ATTGCTTTCA
      1551 GGTAACATAAT ACCAAGTATG TTGTTAGGAC TTGCTAGTGC TTCATGATGC
      1601 TCTAATAATT CGCTAATGAT ACGAGGGTAG CTTTTACCTT CTTTTACTTT
      1651 TNGTGAAAAG GATTCAGATN GTTCAATTTT ATTAATNCTG NGTGCTAATT
20     1701 GCTTTAANGT TTNGATATCA TTATTTTCAC TACCAAATGC AATGGTATCG
      1751 ACACTCATAT AATCNGCGAC TTNAACGGCT AGTTCGGCCA AGGGATCGAC
      1801 CGGCAGGCAG

```

25

Mutant: NT33b

Phenotype: temperature sensitivity

Sequence map: Mutant NT33b is complemented by pMP636,
 30 which contains a 1.8 kb insert of *S. aureus* genomic DNA. A
 partial restriction map is depicted Fig. 38. Database
 searches at both the nucleic acid and peptide levels reveal
 strong peptide-level similarities to the *lepC* gene product,
 encoding signal peptidase I (EC 3.4.99.36) from *B.*
 35 *caldolyticus* (abbreviated as "Bca" in the sequence map).

DNA sequence data: The following DNA sequence data
 represents the sequence of clone pMP636, starting with the
 standard M13 forward and M13 reverse sequencing primers and
 40 applying primer walking strategies to complete the sequence
 contig. The sequences below can be used to design PCR
 primers for the purpose of amplification from genomic DNA
 with subsequent DNA sequencing:

clone pMP636

45 SEQ ID NO. 33

pMP636 Length: 1876 nt

```

      1 TCTGAATGAT CTARACGGAT TAAATTATTT AGCTGGTAAA ACAATCGACG
    51 AAGTTAACAC AAAAGCATTG GAAGGTACAT TATTAGCGCA TACTGATGGT
   101 GGTGTTCCCTA ACATGGTAGT GAACATTCCA CAATTAGATG AAGAAACTTT
5    151 CGGTTACGTC GTATACTTCT TCGAACTTGC TTGTGCAATG AGTGGATACC
    201 AATTAGGCGT AAATCCATTT AACCAACCTG GTGTAGAAGC ATATAAACAA
    251 AACATGTTCG CATTATTAGG TAAACCTGGT TTTGAAGACT TGAAAAAAGA
    301 ATTAGAAGAA CGTTTATAAA ATACATTACT TCAAAGATTA GTGAAGTTTG
    351 AAAAGATAGA ACTAGACGTT AACTATTATA AGCATATTTT CGAGGTTGTC
10   401 ATTACAAATG TAAAAATGTA ATGACAACCT CGTTTTTATT TATATGCAAG
    451 AACTAGGTTA CTAGCTAATG TGACAAGATG TTWAGAGAAA ATTAAAGATA
    501 AAATAATATC TGCCTTACAA TAATATTGTT ATACTACTAG AGACTGATTT
    551 ATTAGCATGA TTACATGTGA ATGTTTCTTT ACTTAGTAAT TAACTTTRTA
    601 ATGTAARAHT AATTATCTTC ADCCAHAAGAA AGGGATTGAT GATTTGTCTG
15   651 WTCMTCAATT AGAAGAATGG TTTGAGATAT KTCGACAGTT TGGTTWTTTA
    701 CCTGGATTTA TATTGTTATA TATTAGAGCT NTAATTCCAG TATTTCTTTT
    751 ARCACTCTAT ATTTTAATTA ACATTCAAGC TTATGGACCT ATTTTAGGTA
    801 TATTGATTAG TTGGCTTGGA TTAATTTCTG GAACATTTAC AGTCTATTTG
    851 ATCTGTAAAC GATTGGTGAA CACTGAGAGG ATGCAGCGAA TTAAACAACG
20   901 TACTGCTGTT CAACGCTTGA TTAGTTTTAT TGATCGCCAA GGATTAATCC
    951 CATTGTTTAT TTTACTTTGT TTTCTTTTAA CGCCAAATAC ATTAATAAAT
   1001 TTTGTAGCGA GTCTATCTCA TATTAGACCT AAATATTATT TCATTGTTTT
   1051 GGCATCATCA AAGTTAGTTT CAACAATTAT TTTAGGTTAT TTAGGTAAGG
   1101 AAATTACTAC AATTTTAACG CATCCTTTAA GARGGATATT AATGTTAGTT
25   1151 GGTGTTGGTT GTATTTTGGA TTGTTGGAAA AAAGTTAGAA CAGCATTTTA
   1201 TGGGATCGAA AAAGGAGTGA CATCGTGAAA AAAGTTGTAA AATATTGAT
   1251 TTCATTGATA CTTGCTATTA TCATTGTACT GTTCGTACAA ACTTTTGTA
   1301 TAGTTGGTCA TGTCATTCCG AATAATGATA TGYMCCCAAC CCTTAACAAA
   1351 GGGGATCGTG TTATTGTWAA TAAAATTAAA GTAACATTTA ATCAATTGAA
30   1401 TAATGGTGAT ATCATAACAT ATAGGCGTGG TAACGGAGAT ATATACTAGT
   1451 CGAATTATTG CCAAACCTGG TCAATCAATG GCGTTTCGTC AGGGACAATT
   1501 ATACCGTGAT GACCGACCGG TTGACGCATC TTATGCCAAG AACAGAAAAA
   1551 TTAAAGATTT TAGTTTGCGC AATTTTAAAG AATTAGGATG GTGATATTAT
   1601 TCCGCCAAAC AATTTTGTTG TGCTAAATGA TCAAGATAAT AACAAGCAGC
35   1651 ATTCAAGACA ATTTGGTTTA ATCGATAAAA AGGATATTAT TGGTAATGTT
   1701 AGTTTACGAT ACTATCCTTT TTCAAAATGG ACTGTTTCACT TCAAATCTTA
   1751 AAAAGAGGTG TCAAAATTGA AAAAAGAAAT ATTGGAATGG ATTATTTCAA
   1801 TTGCAGTCGC TTTTGTCATT TTATTTATAG TAGGTAAATT TATTGTTACG
   1851 CCATATACAA TTAAAGGTGA ATCAAT
40

```

Mutant: NT36

45 **Phenotype: temperature sensitivity**

Sequence map: Mutant NT36 is complemented by pMP109, which contains a 2.7 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 39. Database searches at both the nucleic acid and peptide levels reveal

identity at one end of the pMP109 clone to the *plac* gene from *S. aureus* (Genbank Accession No. M63177), encoding a DNA-directed RNA polymerase (EC 2.7.7.6). Since clone pMP109 does not contain the entire *plac* ORF, the
 5 complementation of mutant NT36 by clone pMP109 is not likely to be due to the presence of this gene. Further analysis of clone pMP109 reveals strong similarity at the peptide level to the *dnaG* gene of *L. monocytogenes* (Genbank Accession No. U13165; published in Lupski et al., 1994,
 10 Gene 151:161-166), encoding DNA primase (EC 2.7.7.-); these similarities also extend to the *dnaG* genes of *L. lactis*, *B. subtilis*, and *E. coli*. The relative size and location of the *dnaG* ORF within clone pMP109 is denoted by an arrow in the sequence map.

15

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP109, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence
 20 contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP109
 25 SEQ ID NO. 34

pMP109 Length: 2687 nt

```

  1  TATGATGATG GTAAAGATCC TAAAGGATTA CCTAAAGCTG ATATTGTTTT
30  51  ACTTGGTATT TCGAGAACTT CAAAGACACC ATTATCTCAG TATTTAGCGC
 101  ATAAGAGTTA CAAAGTTATG AATGTACCGA TTGTACCAGA AGTGACACCG
 151  CCAGATGGCT TATATGATAT TAATCCAAAG AAATGTATCG CACTTAAAAT
 201  AAGTGAAGAA AAATTAAATC GCATTAGAAA AGAGCGACTA AAACAATTAG
 251  GACTAGGTGA CACAGCTCGA TATGCAACAG AAGCACGAAT TCAAGAAGAA
35  301  TTGAATTACT TTGAAGAAAT CGTAAGTGAA ATTGGATGTC CTGTCATTGA
 351  TGTTTCTCAA AAAGCAATCG AAGAAACAGC AAACGATATA ATCCATTATA
 401  TTGAACAAAA TAAATCGAAA TGATTTTCATT TTTGTCGAAA ATTAGGTATA
 451  ATAGTATAAC TAATGCTTAA TAGGTGATTT AATTTGCGAA TAGATCAATC
 501  GATCATTAAAT GAAATAAAAG ATAAAACCGA CATTTTAGAC TTGGTAAGTG
40  551  AATATGTWAA ATTAGAAAAG AGAGGACGCA ATTATATAGG TTTGTGTCCT
 601  TTTCATGATG AAAAGACACC TTCATTTACA GTTTCTGAAG ATAAACAAAT
 651  TTGTCATTGT TTTGGTTGTA AAAAAGGTGG CAATGTTTTT CAATTTACTC
 701  AAGAAATTAA AGACATATTC ATTTGTTGAM GCGGTTAAAG AATTAGGTGG
 751  WTAGRGTTAA TGTTTGCTGT AGRTATTGAG GCAMCACAAT CTTWACTCAA
45  801  ATGTYCAAA TSCTTCTSRV GRTTTACAAA TGATTGACAW TGCATGGRGT

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5 851 TAWTACAAGR ATTTTATTAT TACGCTTTAA CAAAGACAGT CGAAGGCGAA
 901 CAAGCATTAA CGTACTTACA AGAACGTGGT TTTACAGATG CGCTTATTAA
 951 AGAGCGAGGC ATTGGCTTTG CACCCGATAG CTCACATTTT TGTTCATGATT
 10 1001 TTCTTCAAAA AAAGGGTTAC GATATTGAAT TAGCATATGA AGCCGGATTA
 1051 TWATCACGTA ACGAAGAAAA TTTCAGTTAT TTACGATAGA TTYCGAAAYC
 1101 GTATTATGTT YCCTTTGAAA AATGCGCAAG GAAGAATTGT TGGATATTCA
 1151 GGTCGAACAT ATACCGGTCA AGAACCAAAA TACTTAAATA GTCCTGAAAC
 1201 ACCTATCTTT CAAAAAGAA AGTTGTTATA CAACTTAGAT AAAGCGCGTA
 1251 AATCAATTAG AAAATTAGAT GAAATCGTAT TACTAGAAGG TTTTATGGAT
 1301 GTTATAAAAT CTGATACTGC TGGCTTGAAA AACGTTGTTG CAACAATGGG
 1351 TACACAGTTG TCAGATGAAC ATATTACTTT TATACGAAAG TTAACATCAA
 1401 ATATAACATT AATGTTTGAT GGGGATTTTG CGGGTAGTGA AGCAACACTT
 1451 AAAACAGGTY CAAAATTTGT TACAGCAAGG GCTAAATGTR TTTKTTATAC
 1501 AATTGCCATC AGGCATGGAT CCGGATGAAT ACATTGGTAA GTATGGCAAC
 15 1551 GATGCATTTM CTGCTTTTST AAAAAATGAC AAAAAGTCAT TTSCACATTA
 1601 TAAAGTGAGT ATATTAAAAG ATGAAATTGC ACATAATGAC CTTTCATATG
 1651 AACGTTATTT GAAAGAMCTA AGTCATGATA TTTCGCTTAT GAAATCATCG
 1701 ATTTTGCAAC AAAAGGCTTT AAATGATGTT GCACCATTTT TCAATGTTAG
 1751 TCCTGAGCAA TTAGCTAACG AAATACAATT CAATCAAGCA CCAGCCAATT
 20 1801 ATTATCCAGA AGATGAGTAT GCGGTTACA TTGAACCTGA GCCAATTGGT
 1851 ATGGCACAAAT TTGACAATTT GAGCCGTCAA GAAAAAGCGG AGCGAGCATT
 1901 TTTAAAACAT TTAATGAGAG ATAAAGATAC ATTTTAAAT TATTATGAAA
 1951 GTGTTGATAA GGATAACTTC ACAAATCAGC ATTTTAAATA TGTATTCGAA
 2001 GTCTTACATG ATTTTATATG GGAATATGAT CAATATAATA TCAGTGATGC
 25 2051 TGTGCAGTAT GTTAATTCAA ATGAGTTGAG AGAAACACTA ATTAGCTTAG
 2101 AACAATATAA TTTGAATGAC GAACCATATG AAAATGAAAT TGATGATTAT
 2151 GTCAATGTTA TTAATGAAAA AGGACAAGAA ACAATTGAGT CATTGAATCA
 2201 TAAATTAAGG GAAGCTACAA GGATTGGCGA TGTAGAATTA CAAAAATACT
 2251 ATTTACAGCA AATTGTTGCT AAGAATAAAG AACGCATGTA GCATGTGATT
 30 2301 TTAAAGAATA ATACGAATAA TGATTATGTC AAAATGTATA AGGGTAAATG
 2351 ATAGTTACCG CATTTAAACA AACTATTGA AAAATAAATA TTGGGATTAG
 2401 TTCCAATTTG TAAAAATAA TTAATAATAT GGATGAATTA ATTAAGAATT
 2451 TAGTTTAAAA TAGCAATATT GAATAAATTT CGAATGTTCA TATTTAAAAAT
 2501 CGGGAGGCCG TTTCATGTCT GATAACACAG TTAAATTAAT AAAACAAACA
 35 2551 ATTGATCCGA CATTAAACATT AGAAGATGTT AAGAAGCAAT TAATTGAAAA
 2601 AGGTAAAAAA GAGGGTCATT TAAGTCATGA AGAAATTGCT GAAAACTTC
 2651 AGAATTTTGA TATCGACTCT GATCAAATGG ATGATTT

40

Mutant: NT37

Phenotype: temperature sensitivity

45 **Sequence map:** Mutant NT37 is complemented by pMP72, which
 contains a 2.8 kb insert of *S. aureus* genomic DNA. A
 partial restriction map is depicted 40. Database searches
 at both the nucleic acid and peptide levels reveal a strong
 similarity at the peptide level to the *glms* gene of *B.*
subtilis (Genbank Accession No. U21932; published in

Morohoshi, F. et al. *J. Bacteriol.* 175 (1993) 6010-6017), which encodes the protein L-glutamine-D-fructose-6-phosphate amidotransferase (EC 2.6.1.16). The relative location and predicted size of this ORF is designated by an arrow in the sequence map.

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP72, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP72
SEQ ID NO. 35

pMP72 Length: 2800 nt

```

20      1  NTNAATTAAC ATGCGAGGNC ACCCCTTTAT TGCTACTCCA TACTTCTCAT
      51  AAAATCATAT TAACATAACA CCCTTAATTG TCAGACTATT NAAATAAATA
    101  AAACACTTCA TTTTACGCA TTTCTGCCAA ATTAAGATGA AGTAAAAGCT
      151  AAGTCGACCT AAAAAAGCAC CCTTCTAGTC GATTAATCTA AAAGGGGTGC
      201  CATATACTTT AATTTTAATA CATGATTGAT TCTAAAAAAG TGAATTATTC
    251  CACAGTAACT GATTAGCAA GGTTACGTGG TTTATCAACA TCTAAATCTC
      301  TGTGTAATGC TGCATAGTAT GAAATTAATT GTAATGCAAC CACTGATACT
      351  AATGGCGTTA ACAATTCATG TACATGAGGA ATGACATAAG TGTCCGCTTC
      401  TTTTCAAGA CCCTCCATAG AAATAATACA TGGATGTGCA CCACGTGCTA
      451  CTACCTCTTT AACGTTACCA CGAATTGATA AATTAACCTT CTCTTGTTGTT
    501  GCTAAACCTA CAACTGGTGT ACCTTCTTCG ATTAAGGCAA TTGTACCATG
    551  TTTAAGTTCT CCACCAGCAA AACCTTCTGC TTGAATGTAA GAAATTTCTT
      601  TAAGTTTTAA CGCACCTTCT AAACCTACGT TATAGTCAAT AGTACGTCCG
      651  ATAAANAATG CATGCGTGT TGTCTTAAG AAATCTGTAG CAATTTGTTC
      701  CATAATTGGT GCATCGTCAA CAATTGCTTC TATTGCTGTT GTTACTTTTG
    35  751  CTAATTCTCT CAATAAATCA ATATCTGCTT CACGACCATG CTCTTTTGCA
      801  ACGATTTGAG ACAAGAWTGA TAATACTGCA ATTTGTGCAG WATAWGCTTT
      851  TGTAGATGCA ACTGCGAWTT CAGGGACCCG CGTGTAAATA CAATGTGTGG
      901  TCTGCTTCAC GTTGATAAAG TTGAACCTGC AACATTAGTG ATTGTTAATG
      951  AWTATGAMC TAATTTATTA GTTWCAACTA AATACGGCGC GGCTATCTGG
    40  1001  CAGTTTCACC TGATTGAGAA ATATAAACGA ACAATGGTTT TTAAGATAAT
      1051  AATGGCATGT TGTAGACAAA CTCTGATGCA ACGTGTACTT CAGTTGGTAC
      1101  GCCAGCCCAT TTTTCTAAAA ATTCTTTACC TACTAAACCT GCATGGTAGC
      1151  TTGTACCTGC TGCAATAACG TAAATGCGGT CTGCTTCTTT AACATCATTG
      1201  ATGATGTCTT GATCAATTTT CAAGTTACCT TCTGCATCTT GATATCTCTG
    45  1251  AATAATACGA CGCATTACTG CTGGTTGTTC ATGAATTTCT TTTAACATGT
      1301  AGTGTGCATA AACACCTTTT TCAGCATCTG ATGCATCAAT TTCAGCAATA
      1351  TATGAATCAC GTTCTACAAC GTTCCATCT GCATCTTTAA TAATAACTTC

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1401 ATCTTTTTTA ACAATAACGA TTTCATGGTC ATGGRTTCT TTATATTCGC
 1451 TTGTCACTTG TAACATTGCA AGTGCGTCTG ATGCGATAAC ATTGAAACCT
 1501 TCACCAACAC CTAATAATAA TGGTGATTTA TTTTATAGCAA CATAGATTGT
 1551 GCCTTTGHCT TCAGCATCTA ATAAACCTAA TGCATATGAA CCATGTAATA
 5 1601 ATGACACAAC TTTTGTAAT GCTTCTTCAG TTGAAAGTCC TTGATTGAA
 1651 AAGTATTCAA CTAATTGAAC GATAACTTCT GTATCTGTTT CTGAAATGAA
 1701 TGATACACCT TGTAAGTATT CACCTTTTAA CTCTTCATAG TTTTCAATAA
 1751 CACCGTTATG AACTAGAGTA AAACGGCCAT TTGATGATTG ATGTGGATGA
 1801 GAGTTTTTCAT GATTCGGTAC ACCGTGTGTT GCCCAACGTG TGTGACCGAT
 10 1851 TCCAACAGGT CCATTCAAAA TCGCTACTAT CAGCAACTTT ACGTAATTCT
 1901 GCAATACGAC CTTTTTCTTT AAATACAGTT GTATTATCAT YATTTACTAC
 1951 TGCGATACCT GCAGAGTCAT AACCTCTGTA TTCTAATTTT TCTACAACCT
 2001 TTTAATAATA ATTTCTTTGG CATTATCATA GCCAATATAA CCAACAATTC
 2051 CACACATAAC GACATTTTCC TCCATATTGG AATAGTACGS GTAAATATAG
 15 2101 ATTTATTGCC GATAATTTAG ATTGACAATC TGCTTTCATA ATATAAATAG
 2151 GAACATGCTA TCATCGCATT CATCCATAAC AAATTAAGCA TAGTTATTTT
 2201 TACAACATA CAAATTGCTC ACACTGTACT TTCCATATTA ATATTTTTTA
 2251 TATTCAATTT CTGGCGATCT TATTAACCTT GTCCATTAAG TCACCCTAAT
 2301 GTTTTACTTA ATAAGCTAAC GAATGAGCCA CATCCGGGAT AGCATCCGCC
 20 2351 GATCTATTTCG ATCACTATCC TCTTCGTCTA CAAATACATA TATTGCACTC
 2401 TATAAAGGCC ACTCATATAT TAACCTTTAA TCTTCAAATA CAAATATTTA
 2451 TTTGCACAGG CGCTTTAACT GTACTGCCGA ACTTTCCCCC TTTCCATTAA
 2501 TCATTATTGT ACAACGGTGT TGTTTTGTTT TGCAAATATT TTCACAATAA
 2551 AATTTTAAAA ATCCTAAAC AATTTTTTTG TTTTACTTTT TCAAAATATC
 25 2601 TATACTGTCA CATTGATGAC ACTTTATTTA ATTTTGTCAC ATTTATTTTG
 2651 ACAAAGTTGA TTTTGTGTTA TATTGAGTAA CAAGTAACCT CTCTATACAC
 2701 TATATATAGT CACATATATT AAAAAAGAGG TGTAAACATG TCACAAACTG
 2751 AAGAGAAAAA AGGAATTGGT CGTCGTGTTT AAGCATTTGG ATCGACCGCA
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Mutant: NT41/64

Phenotype: temperature sensitivity

35 Sequence map: Mutants NT41 and NT64 are complemented by
 pMP98, which contains a 2.9 kb insert of *S. aureus* genomic
 DNA. A partial restriction map is depicted Fig. 41.
 Database searches at both the nucleic acid and peptide
 levels reveal identity at both the peptide and nucleic acid
 40 levels to the C-terminal fragment of the *pcrA* gene from *S.*
aureus (Genbank Accession No. M63176; published in
 Iordanescu, S.M. et al. *J. Bacteriol.* 171 (1989) 4501-
 4503), encoding DNA helicase (EC 3.6.1.-). Since only a
 small portion of the C-terminal fragment of the helicase
 45 protein is contained within clone pMP98, the *pcrA* gene is
 unlikely to be responsible for restoring a wild-type
 phenotype to mutants NT41 and 64. Further analysis reveals

strong peptide level similarity to the *lig* gene of *E. coli* (Genbank Accession No. M30255; published in Ishino, Y. et al., *Mol. Gen. Genet.* 204 (1986) 1-7), encoding the protein DNA ligase (EC 6.5.1.2). The relative location and predicted size of the ORF encoding the putative *S. aureus* *lig* gene is depicted by an arrow in the sequence map.

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP98, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

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clone pMP98

SEQ ID NO. 36

20 pMP98 Length: 2934 nt

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1  CATGAAATGC AAGAAGAACG TCGTATTTGT TATGTAGCAA TTACAAGGGC
51  TGAAGAGGTG TTATATATCA CTCATGCGAC ATCAAGAATG TTATTTGGTC
101 GCCCTCAGTC AAATATGCCA TCCAGATTTT TAAAGGAAAT TCCAGAATCA
151 CTATTAGAAA ATCATTCAAG TGGCAAACGA CAAACGATAC AACCTAAGGC
201 AAAACCTTTT GCTAAACGCG GATTTAGTCA ACGAACAAACG TCAACGAAAA
251 AACAAGTATT GTCATCTGAT TGGAAATGTAG GTGACAAAGT GATGCATAAA
301 GCCTGGGGAG AAGGCATGGT GAGTAATGTA AACGAGAAAA ATGGCTCAAT
351 CGAACTAGAT ATTATCTTTA AATCACAAGG GCCAAACGT TTGTTAGCGC
401 AATTTGCACC AATTGAAAAA AAGGAGGATT AAGGGATGGC TGATTTATCG
451 TCTCGTGTGA ACGRDTTACA TGATTATTA AATCAATACA GTTATGAATA
501 CTATGTAGAG GATAATCCAT CTGTACCAGA TAGTGAATAT GACAAATTAC
551 TTCTGAACCT GATTAAAATA GAAGAGGAGC ATCCTGAGTA TAAGACTGTA
601 GATTCTCCAA CAGTTAGAGT TGGCGGTGAA GCCCAAGCCT CTTTCAATAA
651 AGTCAACCAT GACACGCCAA TGTTAAGTTT AGGGAATGCA TTTAATGAGG
701 ATGATTTGAG AAAATTCGAC CAACGCATAC GTGAACAAAT TGGCAACGTT
751 GAATATATGT GCGAATTAAA AATTGATGGC TTAGCAGTAT CATTGAAATA
801 TGTTGATGGA TACTTCGTTT AAGGTTTAAC ACGTGGTGAT GGAACAACAG
851 GTTGAAGATA TTACCGRAAA TTTAAAAACA ATTCATGCGA TACCTTTGAA
901 AATGAAAGAA CCATTAAATG TAGAAKTYCG TGGTGAAGCA TATATGCCGA
951 GACGTTTCATT TTTACGATTA AATGAAGAAA AAGAAAAAAA TGATGAGCAG
1001 TTATTTGCAA ATCCAAGAAA CGCTGCTGCG GGATCATTAA GACAGTTAGA
1051 TTCTAAATTA ACGGCAAAAC GAAAGCTAAG CGTATTTATA TATAGTGTCA
1101 ATGATTTTAC TGATTTCAAT GCGCGTTCGC AAAGTGAAGC ATTAGATGAG
1151 TTAGATAAAT TAGGTTTTAC AACGAATAAA AATAGAGCGC GTGTAAATAA
1201 TATCGATGGT GTTTTAGAGT ATATTGAAAA ATGGACAAGC CAAAGAAGAG
1251 TTCATTACCT TATGATATTG ATGGGATTGT TATTAAGGTT AATGATTTAG
1301 ATCAACAGGA TGAGATGGGA TTCACACAAA AATCTCCTAG ATGGGCCATT
1351 GCTTATAAAT TTCCAGCTGA GGAAGTAGTA ACTAAATTAT TAGATATTGA

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1401 ATTAAGTATT GGACGAACAG GTGTAGTCAC ACCTACTGCT ATTTTAGAAC
 1451 CAGTAAAAGT AGCTGGTACA ACTGTATCAA GAGCATCTTT GCACAATGAG
 1501 GATTTAATTC ATGACAGAGA TATTCGAATT GGTGATAGTG TTGTAGTGAA
 1551 AAAAGCAGGT GACATCATAC CTGAAGTTGT ACGTAGTATT CCAGAACGTA
 5 1601 GACCTGAGGA TGCTGTCACA TATCATATGC CAACCCATTG TCCAAGTTGT
 1651 GGACATGAAT TAGTACGTAT TGAAGGCGAA GTTAGCACTT CGTTGCATTA
 1701 ATCCAAAATG CCAAGCACAA CTTGTTGAAG GATTGATTCA CTTTGTATCA
 1751 AGACAAGCCA TGAATATTGA TGGTTTAGGC ACTAAAATTA TTCAACAGCT
 1801 TTATCAAAGC GAATTAATTA AAGATGTTGC TGATATTTTC TATTTAACAG
 10 1851 AAGAAGATTT ATTACCTTTA GACAGAATGG GGCAGAAAAA AGTTGATAAT
 1901 TTATTAGCTG CCATTCAACA AGCTAAGGAC AACTCTTTAG AAAATTTATT
 1951 ATTTGGTCTA GGTATTAGGC ATTTAGGTGT TAAAGCGAGC CAAGTGTKAG
 2001 CAGAAAAATA TGAAACGATA GATCGATTAC TAACGGTAAC TGAAGCGGAA
 2051 TTAGTAGAAT TCATGATATA GGTGATAAAG TAGCGCAATC TGTAGTTACT
 15 2101 TATTTAGCAA ATGAAGATAT TCGTGCTTTA ATTCCATAGG ATTAAAAGAT
 2151 AAACATGTTA ATATGATTTA TGAAGGTATC CAAAACATCA GATATTGAAG
 2201 GACATCCTGA ATTTAGTGGT AAAACGATAG TACTGACTGG TAAGCTACAT
 2251 CCAATGACA CGCAATGAAG CATCTAAATG GCTTGCATCA CCAAGGTGCT
 2301 AAAGTTACAA GTAGCGTTAC TAAAAATACA GATGTCGTTA TTGCTGGTGA
 20 2351 AGATGCAGGT TCAAAATTAA CAAAAGCACA AAGTTTAGGT ATTGAAATTT
 2401 GGACAGAGCA ACAATTTGTA GATAAGCAAA ATGAATTAAA TAGTTAGAGG
 2451 GGTATGTCGA TGAAGCGTAC ATTAGTATTA TTGATTACAG CTATCTTTAT
 2501 ACTCGCTGCT TGTGGTAACC ATAAGGATGA CCAGGCTGGA AAAGATAATC
 2551 AAAACATAA CAATAGTTCA AATCAAGTAA AAGAAATTGC AACGGATAAA
 25 2601 AATGTACAAG GTGATAACTA TCGTACATTG TTACCATTTA AAGAAAGCCA
 2651 GGCAAGAGGA CTTTACAAAG ATAACATGGC AAATAGTTAT AATGGCGGCG
 2701 ACTTTGAAGA TGGTTTATTG AACTTAAGTA AAGAAGTATT TCCAACAGAT
 2751 AAATATTTGT ATCAAGATGG TCAATTTTTG GACAAGAAAA CAATTAATGC
 2801 CTATTTAAAT CCTAAGTATA CAAAACGTGA AATCGATAAA ATGTCTGAAA
 30 2851 AAGATAAAAA AGACAAGAAA GCGAATGAAA ATTTAGGACT TAATCCATCA
 2901 CACGAAGGTG AAACAGATCG ACCTGCAGKC ATGC

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Mutant: NT42

Phenotype: temperature sensitivity

Sequence map: Mutant NT42 is complemented by pMP76, which
 contains a 2.5 kb insert of *S. aureus* genomic DNA. A
 40 partial restriction map is depicted Fig. 42. Database
 searches at both the nucleic acid and peptide levels reveal
 strong similarity at the peptide level to ORFs of unknown
 function in *B. subtilis* (Genbank Accession No. Z38002;
 characterization of the Ipc29D polypeptide is unpublished
 45 as of 1995). Strong similarity is also noted to the SUA5
 protein from the yeast *S. cerevisiae*, which is described as
 being essential for normal growth (published in Na, J.G. et
 al. *Genetics* 131 (1992) 791-801).

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP76, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP76
SEQ ID NO. 37

pMP76 Length: 2515 nt

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15      1 CSYCGGWACC CGGGGATCCT CTAGAGTCGA TCGTTCCAGA ACGTATTCGA
      51 ACTTATAATT ATCCACAAAG CCGTGTAACA GACCATCGTA TAGGTCTAAC
     101 GCTTCAAAAA TTAGGGCAAA TTATGGAAGG CCATTTAGAA GAAATTATAG
     151 ATGCACTGAC TTTATCAGAG CAGACAGATA AATTGAAAGA ACTTAATAAT
     201 GGTGAATTAT AAAGAAAAGT TAGATGAAGC AATTCATTTA ACACAACAAA
20     251 AAGGGTTTGA ACAAACACGA GCTGAATGGT TAATGTTAGA TGTATTTCAA
     301 TGGACGCGTA CGGACTTTGT AGTCCACATG CATGATGATA TGCCGAAAGC
     351 GATGATTATG AAGTTCGACT TAGCATTACA ACGTATGTTA TTAGGGAGAG
     401 CCTATACAGT ATATAGTTGG CTTTGCCTCA TTTTATGGTA GAACGTTTGA
     451 TGTAAACTCA AATTGTTTGA TACCAAGACC TGAAACTGAA GAAGTAATGT
25     501 TGCATTTCTT ACAACAGTTA GAAGATGATG CAACAATCGT AGATATCGGA
     551 ACGGGTAGTG GTGTACTTGC AATTACTTTG AAATGTTGAA AAGCCGGATT
     601 TAAATGTTAT TGCTACTGAT ATTTCACTTG AAGCAATGAA TATGGCTCCG
     651 TAATAAGCT GAGAAGCATC AATCACAAAT ACAATTTTTA ACAGGGGATG
     701 CATTAAAGCC CTTAATTAAT GAAGGTATCA AKTTGAACGG CTTTGATATC
30     751 TAATCCMCCA TATATAGATG AAAAAGATAT GGTACGATG TCTCCMACGG
     801 TTACGARATT CGAACCACAT CAGGCATTGT TTGCAGATAA CCATGGATAT
     851 GCTATTTATG AATCAATCAT GGAAGATTTA CCTCACGTTA TGGAAAAAGG
     901 CAGCCCAGTT GTTTTGA AAA TTGGTTACAA TCAAGGTGAG GCACCTAAAT
     951 CAATAATTTT AAATAAATTT CCTGACAAAA AAATCGACAT TATTAAAGAT
35    1001 ATAAATGGCC ACGATCGAAT CGTCTCATTT AAATGGTAAT TAGAAGTTAT
     1051 GCCTTTGCTA TGATTAGTTA AGTGCATAGC TTTTGGCTTT ATATTATGAT
     1101 AAATAAGAAA GCGGTGATTA AGTTGGATAC TAAAAATTGG GATGTTAGAG
     1151 AATATAATGA AGATTTACAG CAATATCCTA AAATTAATGA AATAAAAGAC
     1201 ATTGTTTTAA ACGGTGGTTT AATAGGTTTA CCAACTGAAA CAGTTTATGG
40    1251 ACTTGCAGCA AATGCGACAG ATGAAGAAGC TGTAGCTAAA ATATATGAAG
     1301 CTAAAGGCCG TCCATCTGAC AATCCGCTTA TTGTTTATAT ACACAGTAAA
     1351 GGTCAATTAA AAGATTTTAC ATATACTTTG GATCCACGCG TAGAAAAGTT
     1401 AATGCAGGCA TTCTGGCCGG GCCCTATTTT GTTTATATTG CCGTTAAAGC
     1451 TAGGCTATCT ATGTCGAAAA GTTTCTGGAG GTTTATCATC AGTTGCTGTT
45    1501 AGAATGCCAA GCCATTCTGT AGGTAGACAA TTATTACAAA TCATAAATGA
     1551 ACCTCTAGCT GCTCCAAGTG CTAATTTAAG TGGTAGACCT TCACCAACAA
     1601 CTTTCAATCA TGTATATCAA GATTTGAATG GCCGTATCGA TGGTATTGTT
     1651 CAAGCTGAAC AAAGTGAAGA AGGATTAGAA AGTACGGTTT TAGATTGCAC
     1701 ATCTTTTCCT TATAAAATTG CAAGACCTGG TTCTATAACA GCAGCAATGA

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1751 TTACAGAAAT AMTTCCGAAT AGTATCGCCC ATGCTGATTA TAATGATACT
1801 GAACAGCCAA TTGCACCAGG TATGAAGTAT AAGCATTACT CAACCCAATA
1851 CACCACTTAC AATTATTACA GATATTGAGA GCAAAATTGG AAATGACGGT
1901 AAAGATTRKW MTTCTATAGC TTTTATTGTG CCGAGTAATA AGGTGGCGTT
5 1951 TATACCAAGT GARSCGCAAT TCATTCAATT ATGTCAGGAT GMCAATGATG
2001 TTAAACAAGC AAGTCATAAT CTTTATGATG TGTACATTG ACTTGATGAA
2051 AATGAAAATA TTTCAGCGGC GTATATATAC GGCTTTGAGC TGAATGATAA
2101 TACAGAAGCA ATTATGAATC GCATGTTAAA AGCTGCAGGT AATCACATTA
2151 TTAAAGGATG TGAACATGTA AGATTTTATT CGTTTGTACA GGTAACACAT
10 2201 GTCGTAGCCC ATTAGCGGGA AGTATTGCAA AAGAGGTTAT GCCAAATCAT
2251 CAATTTGAAT CAAGAGGTAT ATTGCTGTG AACAATCAAG GTGTTTCGAA
2301 TTATGTTGAA GACTTAGTTG AAGAACATCA TTTAGCTGAA ACGACCTTAT
2351 CGCAACAATT TACTGAAGCA GATTTGAAAG CAGATATTAT TTTGACGATG
2401 TCGTATTCGC ACAAAGAATT AATAGAGGCA CACTTTGGTT TGCAAAATCA
15 2451 TGTTTTCACA TTGCATGAAT ATGTAAAAGA AGCAGGAGAA GTTATAGATC
2501 GACCTGCAGG CATGC

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20 Mutant: NT47

Phenotype: temperature sensitivity

Sequence map: Mutant NT47 is complemented by pMP639, which contains a 2.6 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 43, along with open boxes to indicate the percentage of the clone for which DNA sequence has been obtained.. Database searches at both the nucleic acid and peptide levels reveal strong similarity at the peptide level to two hypothetical ORFs of unknown function, one from *K. pneumonia* and one from *Synechocystis* spp. (abbreviated as "Kpn" and "Scy" in the diagram below. Experiments are currently underway to determine which ORF (or both) is an essential gene. The relative orientation and predicted size of these uncharacterized ORFs with respect to the partial restriction map of clone pMP639 are depicted by arrows in the map.

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP639, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

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clone pMP639

SEQ ID NO. 38

pMP639 Length: 2635 nt

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1  ATTCTCTGTG TTGGGGCCCC TGACTAGAGT TGAAAAAAGC TTGTTGCAAG
51  CGCATTTTCA TTCAGTCAAC TACTAGCAAT ATAATATTAT AGACCCTAGG
101 ACATTGATTT ATGTCCCAAG CTCCTTTTAA ATGATGTATA TTTTGTAGAAA
151 TTTAATCTAG ACATAGTTGG AAATAAATAT AAAACATCGT TGCTTAATTT
201 TGTCATAGAA CATTTAATTT AACATCATGA AATTCGTTTT GGCGGTGAAA
251 AAATAATGGA TAATAATGAA AAAGAAAAAA GTAAAAAGTGA ACTATTAGTT
301 GTAACAGGTT TATCTGGCGC AGGTAAATCT TTGGTTATTC AATGTTTGA
351 AGACATGGGA TATTTTTGTG TAGATAATCT ACCACCAGTG TTATTGCCTA
401 AATTTGTAGA GTTGATGGAA CAAGGGAAAT CCATCCTTAA GAAAAAGTGG
451 CAATTGCAAT TGATTTAAGA RGTAAGGAAC TATTTAATTC ATTAGTTGCA
501 GTAGTGGATA AAGTTCAAAA GTTGAAAGTG ACGTCATCAT TGATGTTATG
551 TTTTGTAGAAG CAAGTACTGA AAAATTAATT TCAAGATATA AGGAAACGCG
601 TCCKTGACA TCCTTTGATG GAACAAGGTT AAAAGATCGT TAATCAATGC
651 MATTAATGAT GAGCGAGAGC ATTTGTCTCA AATTAGAAGT ATAGCTAATT
701 TTGTTATAGA TAACTACAAA GTTATCACCT AAAGAATTAA AAGAACGCAT
751 TCGTCGATAC TATGAAGATG AAGAGTTTGA AACTTTTACA ATTAATGTCA
801 CAAGTTTCGG TTTTAAACAT GGGATTGAGA TGGATGCAGA TTTAGTATTT
851 GATGTACGAT TTTTACCAA TCCATATTAT GTAGTAGATT TAAGACCTTT
901 AACAGGATTA GATAAAGACG TTTATAATTA TGTTATGAAA TGGAAAGAGA
951 CGGAGATTTT TCTTTGAAAA ATTAAGTATG TTGTTAGATT TTATGATACC
1001 CGGGTWTAAA AAAGAAGGGA AATCTCAATT AGTAATTGCC ATCGGTTGTA
1051 CGGGTGGGAC AACATCGATC TGTCAGCATT GCAGAACGAC TAGGTWATTA
1101 TCTAAATGAA GTWTTTGAAT ATAATGTTTA TGTGCATCAT AGGGACGCAC
1151 ATATTGAAAG TGGCGAGAAA AAATGAGACA AATAAAAGTT GTACTTATCG
1201 GGTGGTGGCA CTGGCTTATC AGTTATGGCT AGGGGATTAA GAGAATCCCG
1251 AATTGATATT ACGGCGATTG TAACAGTTGC TGATAATGGT GGGAGTACAG
1301 GGAAATCAG AGATGAAATG GATATACCAG CACCAGGAGA CATCAGAAAT
1351 GTGATTGCAG CTTTAAGTGA TTCTGAGTCA GTTTTAAGCC AACTTTTTCA
1401 GTATCGCTTT GAAGAAAATC AAATTAGCGG TCACTCATTG GGTAATTTAT
1451 TAATCGCAGG TATGACTAAT ATTACGAATG ATTTGCGACA TGCCATTAAA
1501 GCATTAAGTA AAATTTTAAA TATTAAAGGT AGAGTCATTC CATCTACAAA
1551 TACAAGTGTG CAATTAATG CTGTTATGGA AGATGGAGAA ATTGTTTTTG
1601 GAGAAACAAA TATTCCTAAA AAACATAAAA AAATTGATCG TGTGTTTTTA
1651 GAACCTAACG ATGTGCAACC AATGGAAGAA GCAATCGATG CTTTAAGGGA
1701 AGCAGATTTA ATCGTTCTTG GACCAGGGTC ATTATATACG AGCGTTATTT
1751 CTAACCTATG TTKTGAATGG TATTTGAGAT GCGTTWATTC ATTCTGATGC
1801 GCCTAAGCTA TATGTTTCTA ATGTGATGAC GCAACCTGGG GAAACAGATG
1851 GTTATAGCGT GAAAGATCAT ATCGATGCGA TTCATAGACA AGCTGGACAA
1901 CCGTTTATTG ATTATGTCAT TTGTAGTACA CAAACTTTCA ATGCTCAAGT
1951 TTTGAAAAAA TATGAAGAAA AACATTCTAA ACCAGTTGAA GTTAATAAGG
2001 CTGAACKTGA AAAAGAAAGC ATAAATGTAA AAACATCTTC AAATTTAGTT
2051 GAAATTTCTG AAAATCATTT AGTAAGACAT AATACTAAAG TGTTATCGAC
2101 AATGATTTAT GACATAGCTT TAGAATTAAT TAGTACTATT CCTTTCGTAC
2151 CAAGTGATAA ACGTAAATAA TATAGAACGT AATCATATTA TGATATGATA
2201 ATAGAGCTGT GAAAAAATG AAAATAGACA GTGGTTCTAA GGTGAATCAT
2251 GTTTTAAATA AGAAAGGAAT GACTGTACGA TGAGCTTTGC ATCAGAAATG
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2301 AAAAATGAAT TAACTAGAAT AGACGTCGAT GAAATGAATG CAAAAGCAGA
 2351 GCTCAGTGCA CTGATTCGAA TGAATGGTGC ACTTAGTCTT TCAAATCAAC
 2401 AATTTGTTAT AAATGTTCAA ACGGAAAATG CAACAACGGC AAGACGTATT
 2451 TATTCGTTGA TTAAACGTGT CTTTAATGTG GAAGTTGAAA TATTAGTCCG
 5 2501 TAAAAAATG AAACCTTAAAA AAAATAATAT TTATATTGT CGTACAAAGA
 2551 TGAAAGCGAA AGAAATTCTT GATGAATTAG GAATTTTAAA AGACGGCATT
 2601 TTTACGCATG AAATTGATCG ACCTGCAGGC ATGCA

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Mutant: NT51

Phenotype: temperature sensitivity

Sequence map: Mutant NT51 is complemented by pMP86, which contains a 1.9 kb insert of *S. aureus* genomic DNA. A

15 partial restriction map is depicted Fig. 44 (there are no apparent restriction sites for EcoR I, Hind III, or BamH I). Database searches at both the nucleic acid and peptide levels reveal strong similarity at the peptide level to an ORF of undetermined function in *H. influenzae* (Genbank
 20 Accession No. U32702):

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP86, starting with the standard M13 forward and M13 reverse sequencing primers and
 25 applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

30 clone pMP86
 SEQ ID NO. 39

pMP86 Length: 1952 nt

35 1 TGCATGTACA GCAGGCTCTA CACAACCGTC GCATGTTTTA GATGCAATGT
 51 TCGAAGATGA GGAGCGATCA AATCATTCGA TTCGATTTAG TTTTAACGAA
 101 TTGACTACTG AAAATGAAAT TAATGCAATT GTAGCTGAAA TTCATAAAAT
 151 ATATTTTAAA TTAAAGGAGG AGTCATAATT GTCAAATAAA GATATAACGT
 201 GTTGTCGTTG GTATGTCAGG CGGTGTAGAT AGTTCTGTAA CAGCCACGT
 40 251 CTTAAAAGAA CAAGGTTATG ATGTCATTGG CATATTTATG AAAAAGTGGG
 301 ATGACACTGA CGAAAATGGC GTATGTACTG CAACTGAAGA TTACAACGAT
 351 GTTATTGAAG TGTGTAATCA AATTGGCATT CCGTATTACG CTGTTAATTT
 401 TGAAAAAGAA TATTGGGATA AAGTCTTTAC GTATTTCTTA GATGAATACA
 451 AAAAAGGTCG TACTCCAAAT CCAGACGTTA TGTGTAATAA AGAAATTAAG
 501 TTTAAAGCCT TTTTAGATCA TGCGATGAAT TTAGGTGCAG ATTATGTAGC
 551 AACAGGACAT TACGCACGCA TACATCGTCA TGAASRTGGT CATGTTGAAA

5 601 TGTTACGTGG TGTAGATAAT AATAAAGATC ARACATACTK CWKGMATGCA
 651 AKTATCTCAA CAACAACTTT CAAAAGTGAT GTTCCCAATT GGCGACATCG
 701 AAAAGAGTGA AGTGCGTCGA ATTGCTGAAG AACAAGGACT TGTTACTGCT
 751 AAGAAAAAAG ATTCTACAGG CATTTGTTTT ATCGGCGAAA AAAACTTTAA
 801 AACATTTTTA TCACAATATT TACCTGCACA ACCGGGTGAT ATGATAACAC
 851 TTGATGGTAA GAAAATGGGT AAACATAGTG GTTTGATGTA TTACACAATA
 901 GGACAAAGAC ATGGATTAGG TATAGGTGGG AGATGGCGAT CCTTGGTTTG
 951 TTGTCGGTAA AAACCTAAAA GATAATGTTT TATATGTWGA ACAAGGATCC
 10 1001 ATCACGATGC ATTATACAGT GATTACTTAA TTGCTTCAGA CTATTCATTT
 1051 GTAAATCCCA GAAGATAATG ACTTAGATCA AGGTTTTGAA TGTACAGCTA
 1101 AATTTAGATA TCGCCAAAAA GATACGAAAG TTTTGTGAA ACGTGAAAAA
 1151 CGACCATGCA CTACGTGTTA CTTTTGCTGA GCCAGTAAGA GCAATCACAC
 1201 CTGGACAAGC AGTTGTTTTT TATCAAGGTG ATGTGTTGTC TTGGTGGTGC
 1251 AACCAATTGAC GATGKTTC AATGAAGG TCAATTAAAT TATGTTGTAT
 15 1301 ANACAATGGC AACAATAAAT TACTTATTTG AAGTTTCNAC GTTGAAAATG
 1351 ACGAAAAGACA GTTTTTGATG AGAATAATTC ATGAGGATAG AGTCTGGGAC
 1401 ATCACAATGT CCTAGGCTCT ACAATGTTAT ATKGGCGGGA CCACAACATA
 1451 GAGAATTTTCG TAAAGAAATT CWACAGGCAA TGCCAGTTGG GGATAACGAA
 1501 TTTAATTTTG TTAATAATAT ATTTCTGTCC CACTCCCTAT GCATGAATCT
 20 1551 AATTATGTAT TCTTATTTTT AAGTACATAA TAGTGGTGGC TAATGTGGAA
 1601 GAACCATTAC ATAATAAACC GTTAATGGTT CTTAAGCATT TYTATTCCAT
 1651 TCCCCGCTTT TCATGAATGA AGATGATATT AGATTATATT TTATTCGTTG
 1701 TTAAGTGATT CGAGACATAC AATTTATCAA GATGTTTATA ATTGATGAGA
 1751 AATGAGGTTC GTAAATGATA GATCAACAAA CAATTTATCA ATACATACAA
 25 1801 AATGGAAAAA TAGAAGAAGC GTTACAAGCA TTGTTCGGAA ATATCGAAGA
 1851 AAATCCTACA ATTATTGAAA ATTATATTAA TGCTGGTATC GTACTTGCTG
 1901 ATGCGAATGA GATTGAAAAG GCAGAGCGTT TTTTCCAAA AGCTTTAACA
 1951 AT

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Mutant: NT52

Phenotype: temperature sensitivity

35 **Sequence map:** Mutant NT52 is complemented by pMP87, which
 contains a 2.3 kb insert of *S. aureus* genomic DNA. A
 partial restriction map is depicted Fig. 45. Database
 searches at both the nucleic acid and peptide levels strong
 peptide-level similarity to the *kimE* gene product, encoding
 40 mevalonate kinase (EC 2.7.1.36), from *M.*
thermoautotrophicum (abbreviated as "Mth" in the sequence
 map.

DNA sequence data: The following DNA sequence data
 45 represents the sequence of clone pMP87, starting with the
 standard M13 forward and M13 reverse sequencing primers and
 applying primer walking strategies to complete the sequence

contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

5 clone pMP87
SEQ ID NO. 40

pMP87 Length: 2273 nt

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10      1 TAACCAATAT TGATAAAACC TTGATGTGTT TCGTGTCAAT GACATACCAT
      51 ATCGACTAGG TACCTTTTTA GAATGTTGAT TAATCACAAC AAATATCATG
     101 GCAAGGTCAT CTTCAAAATG ATTCGATTCA AGTGGAACGG CATATGACGT
     151 CTCATCACTA TACCCTTTTT CCCATTCTGC AAATCCACCA TAAATACTAC
     201 GCGACGCAGA ACCCGAACCA ATTCGCGCCA ATCTCGATAA ATCCTTATCT
     15 251 GACAGCTGCA TGTCTAGCGC TTGATTACAA GCTGCTGCTA AAGCTGCATA
     301 TGC GCTTGCC GATGAAGCCA ACCCTGCTGC TGT TGGTACA AAATTGTCGC
     351 TTTCAATTC TGCATACCAA TCGATGCCAG CTCTATTCT GACAATATCC
     401 ATATATTTTG AAATTTTCTC TAATTCTTTG CCACTAACCT TTTCACCATT
     451 CAACCAAAAT TGATCCTGTG TTAAC TGGTC GTTAAAAGTG ACTTTCGTTT
     20 501 CAGTGTWAAA TTTTCTAAT GTWACAGATA TGCTATTATT CAT TGGGAATG
     551 ATTAGTGCTT CATCTTTTTT ACCCCAATAT TTTATAAGTG CAATATTCGT
     601 ATGTGCACGT GCTTTGCCAC TTTTAATCAA CGCATTAAAC TCCTAAATTC
     651 TCAATCCAAG TATGTGCTGC ACCAGCTTTT TCTACAGCTT TTACAATATT
     701 TTTGCTGTT GGTAAATCTT TGGCAAGCAA TAACATACTT CCACCACGAC
     25 751 CAGCGCCAGT AAGTTTTCCA GCAATCGCAC CATTTTCTTT ACCAATTTTC
     801 ATTAATTGTT CTATTTTATC ATGACTAACT GTCAACGCCT TTAATCCGC
     851 ATGACATTCA TAAAAAATAT CCGCTAAGGS TTCAAAGTTA TGATGTTCAA
     901 TCACATCACT CGCACGTAAA ACTAACTTAC CGATATGTTT TACATGTGAC
     951 ATGTACTGAG GGTCCCTACA AAGTTTATGA ACATCTTCTA CTGCTTGTCT
     30 1001 TGT TGAACCT TTCACACCAG TATCTATAAC AACCATATAG CCGTCTAAAC
     1051 TTAACGTTTT CAACGTTTCA GCATGACCTT TTTGGAACCA AACTGGTTTG
     1101 CCTGATACAA TCGTTTGCCT ATCAATACCA CTTGGTTTAC CATGTGCAAT
     1151 TTGCTCTGCC CAATTAGCCT TTTCAATGAG TTCTTCTTTC GTTAATGATT
     1201 TCCCTAAAAA ATCATAACTT GCACGAACAA AAGCAACCGC GACAGCTGCA
     35 1251 CTCGATCCTA ATCCACGTGA TGGTGGTAAA TTCGTTTGGG TCGTTACTGC
     1301 TAGCGGCTCT GTAATATTAT TTAATTCTAC AAAACGGTTC ACCAAAGAMT
     1351 TAAGATGGTC AGGCGCATCA TATAACATA CCATCGTAAA ACATCGCTTT
     1401 TAATAGAGGA ATAGTTCCCG CTCTCTAAGG TTCTATTAAA ACTTTGATTT
     1451 TAACCGGCGT TAAACGGTAC TGCAATAGCA GGCTCTCCAA ATGTAACAGC
     40 1501 ATGTTCTCCT ATTAATAATA TCTTACCTGT CGATTCCTCC TATCCTTTTC
     1551 TTGTCATGTC AATATCACCT TTTATATTTA TCCTAWACTT GATTCAATTAT
     1601 TTTTATTTAT TAGTAAAAGA CATCATATTC TAAGTKGCAW ACGCATTCGC
     1651 GTTAAATTTT ATTGCAGTCT TTATCTCACA TTATTCATAT TATGTATAAT
     1701 CTTTATTTTG AATTTATATT TGA CT TAACT TGATTAGTAT AAACTAACT
     45 1751 TTCGTTTACT TCAAAGTTTA AATCTTATCG AGTGATATTT CAGATTCTTT
     1801 ATCTTTTTTAT AAAATAGCCC TACAATTTAT AATTTTCCAC CCTAACTATA
     1851 ATACTACAAA TAATAATTGG AATATATAGA TTTACTACTA AAGTATTAGA
     1901 ACATTTCAAT AGAAGGTCGT TTCTTTCATA GTCATACGCA TTATATATAC
     1951 CCTATTCTCA ATCTATTTAA TACGTAAAAC ATGAAATTTT CTTATTAAAT
     50 2001 TTATTATTTT CATCATATCA TTA CT TTTTAA TTTAATGATG TTCAATTTAA

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2051 ATATTAGGTC AATAACATAT TTATGCTTTT TATGGATACT TTCAAAAATA
 2101 ACAGCCCCAA ACGATAACTT GAAAGGGGCT GTTAAATATT TAACTATTGC
 2151 ATTTGATCKA TCATTYTMKW GKWTCYYYSR RTMMYKWKMT CRAAATACGT
 2201 ATCGTATCTT TGCCATTCTT CTTGAGTAAT TGGCGTCATA TTTAATACAC
 5 2251 CGCCAAGATC GACCTGCAGG CAT

10 Mutant: NT53

Phenotype: temperature sensitivity

Sequence map: Mutant NT53 is complemented by pMP143, which contains a 3.0 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 46, along with
 15 open boxes to indicate the percentage of the clone for which DNA sequence has been obtained. Database searches at both the nucleic acid and peptide levels reveal strong similarity at the peptide level to *papS*, encoding poly-A polymerase (EC 2.7.7.19) from *B. subtilis* (Genbank Accession
 20 No. L38424; published in Bower, S. et al. *J. Bacteriol.* 9 (1995) 2572-2575). Also included in this clone is the gene homolog for *birA*, which encodes biotin [acetyl-CoA-carboxylase] ligase and functions as a biotin operon repressor protein.

25 DNA sequence data: The following DNA sequence data represents the sequence of clone pMP143, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to augment the sequence
 30 contigs. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP143

35 SEQ ID NO. 41

pMP143.forward Length: 928 nt

1 TCCTCTAGAG TCGATCAATA TGAGTATTAT TATCAAAAAA TGCTAAATNA
 40 51 GCATAACAAA AGTAAAGGCG AGTAATAATA TGGATAAATC ATTATTTGAA
 101 YAGGCAAGGC CTATATTAGA ACAAATTCAA GACAATGGTT TTNAAGCATA
 151 TTATGTAGGT GGCTCTGTAA GAGATTATGT CATGGGAAGA AATATTCATG
 201 ATATAGATAT CACAACAAGT GCAACGNCGG ATGAAATAGA ATCTATCTTT
 251 AGTCATACGA TACCTGTAGG TAAAGAACAT GGCACGATAA ATGTAGTTTT
 45 301 TAATGATGAA AATTATGAAG TGACAACATT CCGGGCTGAA GAAGATTATG

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351 TCGATCACCG TAGACCAAGT GGTGTTACAT TTGTYCGTGA TTTATACGAR
401 GATTTGCAAC GACGAGATTT CACGATGAAT GCGATAGAAT GGATACAGCA
451 TACAAATTGT ATGATTATTT TGATGGTCAA CAAGATATTA ATAATCGAWT
501 AATAAGAACT GTAGGTATAG CTGAGGAACG TTCCAAGAAG ATGCTTTACG
551 TATGATTCTGA TGTTTAAGGT TCCAGTCACA ATTATCATTT GATATTGCAA
601 CGGAAACATT CGAAGCGATG CGTATACAAA TGGCAGATAT TAAATTTTTTA
651 TCAATTGAGC GTATAGTGAT TGAACAACT AAATTAATGC GAGGTATTAA
701 TGTTGAAAAG AGTTTTAATC ATTTAAAATC GCTGAAAGCA TTTAATTATA
751 TGCCGTATTT CGAACATCTT GATATGAATC AAATTAATGT AACTGAAGCA
801 ATTGATTTAG AATTGTTGAT TGCTATAGTA TCAGTTAAAT TTGATATTAA
851 TTAATCATTG AAGCCTTTAA AGCTAAGTTA ACCGACAAGT TAAAAGATAT
901 CAATCAATAT ATTCAAATTA TGAATGCA

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SEQ ID NO. 42

15 pMP143.reverse Length: 2119 nt

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1 TGCATGCCTG CAGGTCGATC TAATATAGTT TCCGCTAAAT ATAATTGTTG
51 CGGTCGATAT GTTAAGCCAR GTYGATCTAC AGCTTTGCTA TATAAAGACT
101 TCAAGCTGCC ATTATAATTT GTTGTCTGGCT TTTTAAAATC AACTTGCTTA
151 CGATAGATAA TCTGTTTCGAA CTTTTCTGTAC GATTTATCCA ATGGCTTTGC
201 ATCATATTGC CTAACCATCT CAAAGAAAAT ATCATACAAA TCGTATTTCA
251 ACTGTTTACT TAAATAATAT AATTGCTTCA AAGTATCTAA CGGTAACCTTT
301 TCAAATTTTT CAAAAGCTAA TATCATCAAT TTAGCAGTAG TAGCGGCATC
351 TTCGTCAGCT CGATGGGCAT TTGCTAAGGT AATACCATGT GCCTCTGCTA
401 ATTCACTTAA TTGATAGCTT TTATCTGTAG GAAAAGCTAT TTTAAAGATT
451 TCTAGTGTAT CTATAACTTT TTTGGGACGA TATTGAATAT TACAATCTTT
501 AAATGCCTTT TTAATAAAAT TCAAATCAAA ATCTACATTA TGAGCTACAA
551 AAATGCAATC TTTWATCTTA TCGTAGATTT CTTGTGCAAC TTGATTAAAA
601 TATGGCGCTT GTTGTAGCAT ATTTKCTTCA ATGGATGTTA ACGCWTGAAT
651 GAACGGCGGA AWCTCTAAAT TTGTTCTAAT CATAGAATGA TATGTATCAA
701 TAATTTGGTT ATTGCGSACA AACGTTATAC CAATTTGAAT GATATCGTCA
751 AAATCTAATT GGTTGCCTGT TGTTTCCAAA TCCACAACGG CATAGGTTGC
801 CATACCCATA GCTATCTCTC CTTGCTTTAG TGTTAAAAAT CTATATCTGC
851 ACTAATTTAA CGGTGTGATT CACCCGCTTC ATCTCTAACA ATTAGATAGC
901 CATCGTAATC TAAATCAATT GCTTGTCTTT TAAACTGTTT ATCATTTTCT
951 GTAAATAGCA ACGTTCTATT CCAAATATTA GAAGCTGCAG TATATTCTTC
1001 ACGAATTTCA GAAAAAGGTA ACGTTAAAAA TTGATTATAT CTTTTTYCAA
1051 TTTCTTGAAG TAATATCTCT AAAAATTGAT ATCTATCTAA TTwATTTTAA
1101 TCATGTAATT GTATACTTGT TGCTCTATGT CTAATACTTY CATCAAAGTT
1151 TTCTAGTTGT TTGCGTTCAA ATTAATACCT ATACCACATA TTATTGCTTC
1201 TATACCATCC ATTATTAGCA ACCATTTTCAG TTAAGAAACC ACACACTTTA
1251 CCATTATCAA TAAATATATC ATTCGGCCAT TTCATTTTGA CTTTCATCTG
1301 ACTAAAATGT TGAATCGCAT CTCTTATCCC TAATGCAATA AATAAATTAA
1351 ATTTAGATAT CATTGAGAAT GCAACGTTAG GTCTTAACAC GACAGACATC
1401 CAAAGTCCTT GCCCTTTTGA AGAACTCCAA TGTCTATTAA ATCGCCCACG
1451 ACCTTTCGTT TGTTTCATCAG TCAAGATAAA AAATGAAGAT TGATTTCCAA
1501 CAAGTGACTT TTTCGCAGCA AGTTGTGTAG AATCTATTGA ATCGTATACT
1551 TCACTAAAAT CAAACAAAGC AGAACTTTTT GTATATTGGT CTATTATACC
1601 TTGATACCAA ATATCTGGGA GCTGTTGTAA TAAATGCCCT TTATGATTTA
1651 CTGAATCTAT TTTACATCCC TCTAACTTTA ATTGGTCAAT CACTTTTTTT
1701 ACTGCAGTGC GTGGAAATAT TAAGTTGATT CCGCAATGCT TTGTCCAGAA

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1751 TATATAATTC GGTATTATTT TATAGAGTAA TTGAAGTTAC ATCTTGACTA
 1801 TATTTTNACA TGATTATCCA CCCATTTCAA AATTNCAGTT TCTNCGTTGC
 1851 TTACTTTACC TGTNACAATC GCTATCTCAA TTTGTCTTAG CACATCTTTT
 1901 AACCACGGAC CACTTTTGGC ATTTAAATGT GCCATAAGTA CACCGCCATT
 5 1951 AACCATCATG TCTTTNCTAT TATGCATAGG TAAACGATGT AATGTTTCAT
 2001 CAATCGTTTG AAGGTTAACG CTTAATGGTT CATGTCCTTG GTATCATAAC
 2051 GCCTGTNTCA AGCGTTCTNC AANCATGTAC AGTTNTTCAA TGTGGNGTGT
 2101 CCGNATTAAC GCTATTCAA

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Mutant: NT54

Phenotype: temperature sensitivity

15 **Sequence map:** Mutant NT54 is complemented by pMP145, which
 contains a 3.1 kb insert of *S. aureus* genomic DNA. A
 partial restriction map is depicted Fig. 47, along with
 open boxes to indicate the percentage of the clone for
 which DNA sequence has been obtained. Database searches at
 20 both the nucleic acid and peptide levels reveal identity at
 the nucleic acid level and peptide level to the C-terminal
 portion of the *pbp4* gene, encoding D,D-carboxy peptidase
 (EC 3.4.16.4) from *S. aureus* (Genbank Accession No. U29454;
 unpublished as of July, 1995). Since clone pMP146 does not
 25 contain the complete *Pbp4* ORF, this gene is unlikely to be
 responsible for restoring mutant NT54 to a wild-type
 phenotype. Cross complementation with clone pMP91, which
 contains a 5.2 kb insert of *S. aureus* genomic DNA, reveals
 that only 800 additional base pairs downstream (3' to) the
 30 *Pbp4* ORF are necessary for complementation (data not
 shown). DNA sequence of this region reveals strong
 similarity at the nucleic acid and peptide levels to the
tagD gene, encoding glycerol-3-phosphate cytidyl
 transferase (EC 2.7.7.39), from *B. subtilis* (Genbank
 35 Accession No. M57497; published in Mael, C. et al., J.
 Gen. Microbiol. 137 (1991) 929-941). The *tagD* gene of *B.*
subtilis has been reported to be an essential gene and is
 therefore likely to be a good candidate for screen
 development. The relative size and location of the *TagD*
 40 ORF with respect to clone pMP145 is depicted by an arrow in
 the restriction map.

DNA sequence data: The following DNA sequence data
 represents the sequence of the right-most portion of clone

pMP145, starting with the standard M13 reverse sequencing primer and applying primer walking strategies to complete the sequence contig. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP145

SEQ ID NO. 43

10 pMP145 Length: 1407 nt

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      1 TTCACAGTGT TGTCGGGATA CGATATAGTA CACTGTACAG TACGNTGGAG
     51 ATTTATTAGA TTTTCACAGA ATTNTGAAAA TAAGACNACG GGTCATGGAA
    101 ATGTTACTAT TACCTGAACA AAGGCTATTA TATAGTGATA TGGTTGNTCG
    15 151 TATTTTATTC AATAATTCAT TAAAATATTA TATGAACGAA CACCCAGCAG
    201 TAACGCACAC GACAATTCAA CTCGTAAAAG ACTATATTAT GTCTATGCAG
    251 CATTCTGATT ATGTATCGCA AAACATGTTT GACATTATAA ATACAGTTGA
    301 ATTTATTGGT GAGAATTGGG ATAGAGAAAT ATACGAATTG TGGCGACCAA
    351 CATTAATTCA AGTGGGCATT AATAGGCCGA CTTATAAAAA ATTCTTGATA
    20 401 CAACTTAAAG GGAGAAAGTT TGCACATCGA ACAAATCAA TGTTAAAACG
    451 ATAACGTGTA CATTGATGAC CATAAACTGC AATCCTATGA TGTGACAATA
    501 TGAGGAGGAT AACTTAATGA AACGTGTAAT AACATATGGC ACATATGACT
    551 TACTTCACCTA TGGTCATATC GAATTGCTTC GTCGTGCAAG AGAGATGGGC
    601 GATTATTTAA TAGTAGCATT ATCAACAGAT GAATTTAATC AAATTAACA
    25 651 TAAAAAATCT TATTATGATT ATGAACAACG AAAAATGATG CTTGAATCAA
    701 TACGCTATGT CRTATTTAGT CATTCCAGAA AAGGGCTGGG GACAAAAAGA
    751 AGACGATGTC GAAAAATTTG ATGTAGATGT TTTTGTATG GGACATGACT
    801 GGGAAAGTGA ATTCGACTTC TTAAAGGATA AATGTGAAGT CATTTATTTA
    851 AAACGTACAG AAGGCATTTT GACGACTAAA ATCAAACAAG AATTATATGG
    30 901 TAAAGATGCT AAATAAATTA TATAGAACTA TCGATACTAA ACGATAAATT
    951 AACTTAGGTT ATTATAAAAT AAATATAAAA CGGACAAGTT TCGCAGCTTT
   1001 ATAATGTGCA ACTTGTCCTG TTTTAGTATG TTTTATTTTC TTTTCTTAAA
   1051 TAAACGATTG ATTATCATAT GAACAATAAG TGCTAATCCA GCGACAAGGC
   1101 ATGTACCACC AATGATAGTG AATAATGGAT GTTCTTCCCA CATACTTTTA
   35 1151 GCAACAGTAT TTGCCTTTTG AATAATTGGC TGATGAACTT CTACAGTTGG
   1201 AGGTCCATAA TCTTTATTAA TAAATCTCTT TGGATAGTCC GCGTGTAATT
   1251 TACCATCTTC GACTACAAGT TTATAATCTT TTTTACTAAA ATCACTTGGT
   1301 AAAACATCGT AAAGATCATT TTCAACATAA TATTTCTTAC CATTTATCCT
   1351 TTGCTCACCT TTAGACAATA TTTTACATA TTTTACTGA TCAAATGAVC
   40 1401 GTTCCAT

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45 Mutant: NT55

Phenotype: temperature sensitivity

Sequence map: Mutant NT55 is complemented by pMP92, which contains a 2.0 kb insert of *S. aureus* genomic DNA. A

partial restriction map is depicted Fig. 48. Database searches at both the nucleic acid and peptide levels reveal strong peptide-level similarity to the *nadE* gene product, encoding the nitrogen regulatory protein NH₃-dependent NAD synthetase (EC 6.3.5.1), from *E. coli* (Genbank Accession No. M15328; published in Allibert, P. et al. *J. Bacteriol.* 169 (1987) 260-271).

DNA sequence data: The following DNA sequence data represents the sequence of clone pMP92, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to complete the sequence contig. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP92

SEQ ID NO. 44

20 pMP92 Length: 1996 nt

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      1 TCCTCTAGAG TCGATCGTAT TAAATTATCA AATAACGCTG AAAAGGTTAC
     51 GACGCCAGGT AAGAAAAATG TATATCGCAT TATAACAAG AAAACAGGTA
    101 AGGCAGAAGG CGATTATATT ACTTTGGAAA ATGAAAATCC ATACGATGAA
    25 151 CAACCTTTAA AATTATTCCA TCCAGTGCAT ACTTATAAAA TGAAATTTAT
     201 AAAATCTTTC GAAGCCATTG ATTTGCATCA TAATATTTAT GAAAATGGTA
     251 AATTAGTATA TCAAATGCCA ACAGAAGATG AATCACGTGA ATATTTAGCA
     301 CTAGGATTAC AATCTATTTG GGATGAAAAT AAGCGTTTCC TGAATCCACA
     351 AGAATATCCA GTCGATTTAA GCAAGGCATG TTGGGATAAT AAACATAAAC
    30 401 GTATTTTTGA AGTTGCGGAA CACGTTAAGG AGATGGAAGA AGATAATGAG
     451 TAAATTACAA GACGTTATTG TACAAGAAAT GAAAGTGAAA AAGCGTATCG
     501 ATAGTGCTGA AGAAATTATG GAATTAAAGC AATTTATAAA AAATTATGTA
     551 CAATCACATT CATTTATAAA ATCTTTAGTG TTAGGTATTT CAGGAGGACA
     601 GGATTCTACA TTAGTTGGAA AACTAGTACA AATGTCTGTT AACGAATTAC
    35 651 GTGAAGAAGG CATTGATTGT ACGTTTATTG CAGTTAAATT ACCTTATGGA
     701 GTTCAAAAAG ATGCTGATGA AGTTGAGCAA GCTTTGCGAT TCATTGAACC
     751 AGATGAAATA GTAACAGTCA ATATTAAGCC TGCAGTTGAT CAAAGTGTGC
     801 AATCATTAAG AGAAGCCGGT ATTGTTCTTA CAGATTTCCA AAAAGGAAAT
     851 GAAAAAGCGC GTGAACGTAT GAAAGTACAA TTTTCAATTG CTTCAAACCG
    40 901 ACAAGGTATT GTAGTAGGAA CAGATCATTC AGCTGAAAAT ATAAGTGGGT
     951 TTTATACGAA GTACGGTGAT GGTGCTGCAG ATATCGCACC TATATTTGGT
    1001 TTGAATAAAC GACAAGGTCG TCAATTATTA GCGTATCTTG GTGCGCCAAA
    1051 GGAATTATAT GAAAAAACGC CAACTGCTGA TTTAGAAGAT GATAAACAC
    1101 AGCTTCCAGA TGAAGATGCA TTAGGTGTAA CTTATGAGGC GATTGATAAT
    45 1151 TATTTAGAAG GTAAGCCAGT TACGCCAGAA GAACAAAAAG TAATTGAAAA
    1201 TCATTATATA CGAAATGCAC ACAAACGTGA ACTTGCATAT ACAAGATACA
    1251 CGTGGCCAAA ATCCTAATTT AATTTTTTCT TCTAACGTGT GACTTAAATT
    1301 AAATATGAGT TAGAATTAAT AACATTAAAC CACATTCAGC TAGACTACTT

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1351 CAGTGTATAA ATTGAAAGTG TATGAACTAA AGTAAGTATG TTCATTTGAG
 1401 AATAAATTTT TATTTATGAC AAATTCGCTA TTTATTTATG AGAGTTTTTCG
 1451 TACTATATTA TATTAATATG CATTCAATTA GGTTAGGTTG AAGCAGTTTG
 1501 GTATTTAAAG TGTAATTGAA AGAGAGTGGG GCGCCTTATG TCATTCGTAA
 5 1551 CAGAAAATCC ATGGTTAATG GTACTAACTA TATTTATCAT TAACGTTTGT
 1601 TATGTAACGT TTTTAACGAT GCGAACAATT TTAACGTTGA AAGGTTATCG
 1651 TTATATTGCT GCATCAGTTA GTTTTTTAGA AGTATTAGTT TATATCGTTG
 1701 GTTTAGGTTT GGTTATGTCT AATTTAGACC ATATTCAAAA TATTATTGCC
 1751 TACGCATTG GTTTTTCAAT AGGTATCATT GTTGGTATGA AAATAGAAGA
 10 1801 AAAACTGGCA TTAGGTTATA CAGTTGTAAA TGTAACCTCA GCAGAATATG
 1851 AGTTAGATT ACCGAATGAA CTTCGAAATT TAGGATATGG CGTTACGCAC
 1901 TATGCTGCGT TTGGTAGAGA TGGTAGTCGT ATGGTGATGC AAATTTTAAC
 1951 ACCAAGAAAA TATGAACGTA AATTGATGGA TACGATAAAA AATTTA

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Mutant: NT57

Phenotype: temperature sensitivity

20 **Sequence map:** Mutant NT57 is complemented by pMP94, which
 contains a 3.6 kb insert of *S. aureus* genomic DNA. A
 partial restriction map is depicted Fig. 49, along with
 open boxes to indicate the percentage of the clone for
 which DNA sequence has been obtained.. Database searches
 25 at both the nucleic acid and peptide levels reveal
 significant similarity at the peptide level to the gap
 gene, encoding glyceraldehyde-3-phosphate dehydrogenase (EC
 1.2.1.12), from a number of prokaryotes and eukaryotes
 (e.g. Genbank Accession No. M24493, for the corresponding
 30 gene from *B. stearothermophilus*; published in Branlandt, C.
 et al., 1989, *Gene* 75:145-155). From the opposite sequence
 contig, a strong peptide-level similarity is noted to the
 dnaB gene product, encoding an essential protein involved
 in the initiation of DNA replication, from *B. subtilis*
 35 (Genbank Accession No. M15183; published in Hoshino, T. et
 al. *Proc. Natl. Acad. Sci. USA* 84 (1987) 653 - 657). Also
 of significance is the similarity of a subclone sequence to
 an ORF of unknown function, conserved among prokaryotes
 including *E. coli*, *M. leprae*, *C. acetobutylicum*, *H.*
 40 *influenzae* and *B. subtilis* (e.g. "orf 168" from Genbank
 Accession No. D28752). The relative orientations and
 predicted sizes of the ORFs identified in this entry are
 denoted by arrows in the restriction map.

DNA sequence data: The following DNA sequence data represents the partial sequence of clone pMP94, starting with the standard M13 forward and M13 reverse sequencing primers and applying primer walking strategies to augment the sequence contigs as well as obtain subclone sequence data. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP94

SEQ ID NO. 45

pMP94.forward Length: 1017 nt

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15      1  CTTYGARCTC GGTACCCGGG GMTCCTCTAR AGTCGATCTT TATACTCTTG
      51  TAACACATTT AAGTCTTCAT CAATCATAGC ATTCGTTAAT TCAGCTCGAT
     101  GCGCTTCCAA AAATTGCTTA ACATCTGGGT CATWGATGTC TCCTGATTTT
     151  ATCTTTTCTA TTCTTTTTTC AAAGTCCTGC GACGTGTTAA TTATACTTTT
     201  AAATTGCTTC ATTATTGACT GTCCTCCTCC CATTTTTTAG ATAATTTATC
20     251  TAGAAATGCT TGTCGATCTT GCTCTAATTG TTGATCATCT ACGCTATTAT
     301  CTTTAGCCGA ATCTTCTTCA CTAGGTTTAT CTCTATTTTC TAACCATTTA
     351  GGTGTTTTTT CTTTTGAAAT ACGATTACGC TGCCCATAGT ATGAACCACG
     401  CTTTGGGTAA TTTCCGCTAG AACCCTCATT TTTAGGTTGA TTAACTTTTT
     451  TAGCGTAATT ATATGCTTCT TTAGCTGTCT TAATACCTTT TTTCTTCCAA
25     501  TTTGATGCTA TTTCCAAAAT ATACGCTTTA GGAAGTTTCA TATCTTCTTT
     551  TAACATGACA AATTGCAACA AAATATTAAT GACGCCAAAA GACATTTTTT
     601  CACGTTTCAA TTAATTCTTC AACCATTGTC TTTTGCGATA TAGTTGGTYC
     651  TGATTCAAGT CAAGAAGCTA ACATATCAAT TGGACTCGTT TGTTCAAGTA
     701  ACTCAAACCA TTCATCACTT TGTGGCTTTG GATTCACCTC TGAAGATTG
30     751  CCCGCCGAAG ATGATGTAGC AGGAGATTTC ACCTGTAATT TAGGCATTG
     801  ATTTTCGTGT TCCATTAAGT AATACGAGCG TGCTTGTTTA CGCATTTCCT
     851  CAAAGGATAA CTGTTGTCCA CTTGTAATTG AATTTAAAAT AACATGCTTC
     901  ATGCCATCTG CTGTTAAACC ATATAAATCN CGAATTGTGT TATTAAACCC
     951  TTGCATCTTG GTAACAATGT CTTGACTAAT AAATGTTTAC CTAACATTGT
35    1001  CTCCACATTT CNANTCC

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SEQ ID NO. 46

pMP94.reverse Length: 1035 nt

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40      1  TGCATGCCTG CAGGTCGATC AAGGGGTGCT TTTAATGTCA AMGAATATTG
     51  CAATTRATGG TATGGGTAGA ATTGGAAGAA TGGTATTACG TATTGCATTA
    101  CAAAATAAAA ATTTAAATGT AGTAGCGATA AATGCTAGTT ATCCACCCGA
    151  AACAATTGCA CATTTAATCA ATTACGATAC GACACATGGA AAATATAATC
    201  TAAAAGTTGA ACCGATTGAA AATGGATTGC AAGTTGGAGA TCATAAAATT
45    251  AAATTGGTTG CTGATCGCAA TCCTGAAAAC TTGCCATGGA AAGAATTAGA
     301  TATCGATATT GCTATAGATG CAACTGGTAA ATTTAATCAT GGTGATAAAG
     351  CCATCGCACA TATTAAAGCA GGTGCCAAAA AAGTTTTGTT AACTGGTCCT
     401  TCAAAGGTG GACATGTTCA AATGGTAGTT AAAGGCGTAA ATGATAACCA
     451  ATTAGATATA GAAGCATTG ACATTTTTFAG TAATGCTTCA TGTACTACTA

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501  ATTGCATTGG TCCAGTTGCA AAAGTTTTAA ATAATCAGTT TGGGAATAGT
551  TAATGGTTTA ATGACTACTG TTCACGCTAT TACAAATGAC CAAAAAATA
601  TTGATAATCC MCATAAAGAT TTAAGACGTG CACGTTTCATG TWATGAAAGC
651  ATTATTCCTA CTTCTACTGG TCGGCGGAAA GCTTTAAAAAG AAGTATTACC
5    701  AGAATTAGAA GGTAAATTAC ACGGCATGGC ATTACGTTGT ACCAACAAAG
751  AATGTATCGC TCGTTGATTT AGTTGTTGAT TTAGAAAAAG AAGTAACTGC
801  AGAAGAANTA AACCAAGCTT TTGAAAATGC AGGTTTAGAA GGTATCATAG
851  AANTCGAACA TCACCACTAG TGTCTGTTGA TTTTAATACT AATCCCAATT
901  CAGCTATTAT TGATGCCAAA CCACNATGTC ATGTTCCGGG AAATAAGTAA
10   951  ANTTATTGCT TGGTATGAAN ATGAATGGGG TTATTCCAAT AAATTGTTAA
1001 NNTTGCNGAA CAAATTGGAC NCTTTGGANT CCAAA

```

SEQ ID NO. 47

pMP94.subclone Length: 483 nt

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15   1  CTCCGTTTGT TTTGCTTAA AATCCCTTGC ATCGATGCTA ACAATTGATC
51   51  AACATCTTTA AATTCTTTAT AGACTGATGC AAATCTAACA TATGAACTT
101  101  GATCAACATG CATTAACAAG TTCATAACGT GTTCACCTAT ATCTCGTGAA
151  151  GACACTTCCG TATGACCTTC ATCTCGTAAT TGCCATTCAA CCTTGTTAGT
20   201  TATGACTTCA AGTTGTTGAT ATCTAACTGG TCGTTTCTCA CAAGAACGCA
251  251  CAAGTCCATT AAGTTATCTT TTCTCTTGAA AACTGCTCTC TTGTGCCATC
301  301  TTTTTCACA ACTATAAGCT GACTAACTTC GATATGNTTC AAATGTTAGT
351  351  GGAAACGTTG TTTCCACAAT TTTACATTTC TCTTCGTCTT CCGAAATGGC
401  401  ATTTAATTCA TCGGGCATGC CTTGAATCTA CAACTTTAGA ATTGTGTTAG
25   451  AATTACATTT CGGGCATTTC ATTACATCAC CTC

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30 Mutant: NT68

Phenotype: temperature sensitivity

Sequence map: Mutant NT68 is complemented by pMP163, which contains a 5.8 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 50. Database searches at both the nucleic acid and peptide levels reveal strong peptide-level similarities to the *dnaE* gene, encoding DNA polymerase III alpha subunit (EC 2.7.7.7), from Gram-negative bacteria such as *S. typhimurium* (Genbank Accession No. M29701; published in Lancey, E.D., et al. *J. Bacteriol.* 171 (1989) 5581 - 5586). This mutant is distinct from NT28, described previously as having a mutation in the *polC* gene which also encodes an alpha subunit of DNA polymerase III (found so far in Gram-positive bacteria). Although *dnaE* and *polC* putatively encode proteins of the same enzymatic function, in *S. aureus* these two genes are quite distinct and may or may not encode proteins of redundant function; since the DNA

sequences of each are less than 65% identical, they are confirmed as being two distinct essential genes.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP163, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP163
SEQ ID NO. 48

15

pMP163 Length: 5718 nt

```

      1 CTCGGTACCC GGGGATCGTC ATGGAATACC GGAATATTAG TTTCTTTTTT
    51 CAATCGTTCT TCAATTTCAA AACACGTGG TGCCGAAATA TCCTCTAAAT
  20 101 TAATACCACC ATAATTAGGT TCTAACAACT TAACTGTTTT AATGATTTCT
    151 TCGGTATCAG TTGTATTTAA CGCAATAGGC ACCCCATTGA TACCAGCGAA
    201 GCTTTTGAAT AATACTGCTT TACCTTCCAT TACAGGAATA CTTGCTTCAG
    251 GTCCAATGTT ACCTAAACCT AATACCGCTG TTCCATCAGT AATAACTGCA
    301 ACTGTATTTC CTTTAATTGT GTAATCATAT ACTTTTCTTT TATCTTCATA
  25 351 AATATCTTTA CACGGTTCAG CAACGCCAGG TGAGTATGCT AAACCTTAATT
    401 CCTCTTTATT AGTAACCTTT ACATTTGGTT TAACTTCTAA TTTACCTTGA
    451 TTACGTTTGT GCATTTCCAA TGCTTCATCT CTTAATGACA TGAAATCAGC
    501 CCCTAATTCA ATATTTATTT TTAAAAATA ACTTGGATAA AACGCATTAC
    551 ATTATAAAAG TAAAAATATT GGGTAATCTG AATGARTAAG AATTTATGGT
  30 601 TTTGATTATG TAAACAAAT AGCGATAAAC GATAATAAAA TAATATTTAT
    651 AAAGATACAT TAAACCATAC TATCTAAAGA TATACCTTTA ATTATTATAA
    701 TGGATAGCAA AAACCAATAT ATCAAAAAGT TATTATTTTT CCGCACGATA
    751 TATCGACAAA ATTCTTTACT CAATTTATGT ATACTGCTTT TTGTGCTAAT
    801 TATTCTTATG GATTAATCAA TAATGTAAAG TGAAACTCAT AAAAATAATA
  35 851 AGCATAAAAA ACTAATATAA ACGCAAACCTG ATGGTTAAAA AATATCTAAC
    901 CATCAGTTTA CTATATCATA ATTTATTAGT TGATAAAAAGT TATATAAGCC
    951 TAATATCACT AGGGTTAAAG GGATTGTATA AAATTATTAA ACATACTATC
  1001 TTTTGGATTA ATATAGCCTA AAGTAGTCAT TTGTTTAATC GTTTCATCAT
  1051 AAAAGGATAA CACAACATCA TTAGCATTCT CTTTCGTAGC TTAAATCATC
  40 1101 TCTTCAAACA TATCTATTTG TGATTTATTT CTAATTATAA TTTGTTTGGC
    1151 AAATGCTAAT TTTTGTTCTT CAAAAGTGGC TAATGTCTGA ATCTCATTTA
    1201 TAATTAGTTG ACGTTGTTGC TTTCTATGGT CAAATTTCCC GCTAACTATA
    1251 AACAAAGTCAT TATGTGATAA CAACTCTTCG TACTTTTFAA ACTGATTAGG
    1301 GAAAATCACA CCATCTAAAG TTTCAATGCC ATCATTTAAT GTTGACGAAT
  45 1351 GCCATATTTT GACCATTTTT AGTTCGAATT TGTTTAACTT TATCAAACCTG
    1401 TACTAATATA GGTTTATAAT TCTGCGCGTT ACTCAATTTA AATATCGTTA
    1451 AATATTGTTT GGCAACAAAC TTTTATCTA CTGGGTGTTG CGAAACATAA
    1501 AATCCTAAAT ATTCTTTTTT GTACTGACTA ATAAGTGCAT CAGGCAATTC

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	1551	TTCTTTATCT	TCATACATCT	GTTTTGGCGT	TAAAAATATCA	AATAAAAAAC
	1601	CATCTTGTTT	AATGTTTAAA	TCGCCATCCA	ACACTTGATC	AATAGCTTGC
	1651	AACAACGTTG	AACGTGTTTT	ACCAAAAAGCA	TCAAACGCTC	CCACTAAAAT
	1701	CAGTGCTTCA	AGTAACTTTC	TCGTTWTGAM	YCTCTTCGGT	ATACGTCTAG
5	1751	CAWAATCAAA	GAAATCTTTA	AATTTGCCGT	TCTGATAACG	TTCATCAACA
	1801	ATCACTTTCA	CACTTTGATA	ACCAACACCT	TTAATTGTAC	CAATTGATAA
	1851	ATAAATGCCT	TCTTGGGAAG	GTTTATAAAA	CCAATGACTT	TCGTTAATGT
	1901	TCGGTGGCAA	TATAGTGATA	CCTTGTTTTT	TTGCTTCTTC	TATCATTTGA
	1951	GCAGTTTTCT	TCTCACTTCC	AATAACATTA	CTTAAAAATAT	TTGCGTAAAA
10	2001	ATAATTTGGA	TAATGGACTT	TTAAAAAGCT	CATAATGTAT	GCAATTTTAG
	2051	AATAGCTGAC	AGCATGTGCT	CTAGGAAAAC	CATAATCAGC	AAATTCAGA
	2101	ATCAAATCAA	ATATTTGCTT	ACTAATGTCT	TCGTGATAAC	CATTTTGCTT
	2151	TGSMCCTTCT	ATAAAATGTT	GACGCTCACT	TTCAAGAACA	GCTCTATTTT
	2201	TTTTACTCAT	TGCTCTTCTT	AAAATATCCG	CTTCACCATA	ACTGAAGTTT
15	2251	GCAAATGTGC	TCGCTATTTG	CATAATTTGC	TCTTGATAAA	TAATAACACC
	2301	GTAAGTATTT	TTAATATAG	GTTCTAAATG	CGGATGTAAA	TATTGAACCT
	2351	TGCTTGGATC	ATGTCTTCTT	GTAATGTAAG	TTGGAATTTT	TTCCATTGGA
	2401	CCTGGTCTAT	ACAAAGAAGT	TACAGCAACA	ATATCTTCAA	AGTGTTCCGG
	2451	CTTTAATTTT	TTTAATACAC	TTCTTACACC	GTCAGACTCT	AATTGGAATA
20	2501	TGCCAGTCGT	ATCTCCTTGC	GACAACAATT	CAAACACTTT	TTGATCATCA
	2551	AACGGAATCT	TTTCGATATC	AATATTAATA	CCTAAATCTT	TTTTGACTTG
	2601	TGTTAAGATT	TGATGAATAA	TCGATAAGTT	TCTCAACCCCT	AGAAAATCTA
	2651	TTTTTAATAA	CCCAATACGT	YCGGCTTCAG	TCATTGTCCA	TTGCGTTAAT
	2701	AATCCTGTAT	CCCCTTTTCGT	TAAAGGGGCA	TATTCATATA	ATGGATGGTC
25	2751	ATTAATAATA	ATYCCTGCCG	CATGTGTAGA	TGTATGTCTT	GGTAAACCTT
	2801	CTAACTTTTT	ACAAATACTG	AACCAGCGTT	CATGTCGATG	GTTTCGATGT
	2851	ACAAACTCTT	TAAAAATCGT	AATTTGATAT	GCTTCATCAA	GTGTAATTCC
	2901	TAATTTATGT	GGGATTAAAC	TTGAAAAATTT	CATTTAATGT	AACCTCATCA
	2951	AACCCCATAA	TTCTTCCAAC	ATCTCTAGCA	ACTGCTCTTG	CAAGCAGATG
30	3001	AMCGAAAGTC	ACAATTCCAG	ATACATGTAG	CTCGCCATAT	TTTTCTTGGA
	3051	CGTACTGAAT	GACCCCTTCT	CGGCGTGTAT	CTTCAAAGTC	AATATCAATA
	3101	TCAGGCATTG	TTACACKTTC	TGGGTTTTAAA	AAACGTTCAA	ATAATAGATT
	3151	GAATTTAATA	GGATCAATCG	TTGTAATTCC	CAATAAATAA	CTGACCAGTG
	3201	AGCCAGCTGA	AGAACCACGA	CCAGGACCTA	CCATCACATC	ATTGTTTTTC
35	3251	GCATAATGGA	TTAAATCACT	WACTATTAAG	AAATAATCTT	CAAAACCCAT
	3301	ATTAGTAATA	ACTTTATACT	CATATTTCAA	TCGCTCTAAA	TAGACGTCAT
	3351	AATTAAGTTC	TAATTTTTTTC	AATTGTGTAA	CTAAGACACG	CCACAAATAT
	3401	TTTTTAGCTG	ATTCATCATT	AGGTGTCTCA	TATTGAGGAA	GTAGAGATTG
	3451	ATGATATTTT	AATTCTGCAT	CACACTTTTT	AGCTATAACA	TCAACCTGCG
40	3501	TTAAATATTT	CTTGGTTAAT	ATCTAATTGA	TTAATTTCCCT	TTTTCAGTTA
	3551	AAAAATGTGC	ACCAAAATCT	TTCTTGATCA	TGAATTAAGT	CTAATTTTGT
	3601	ATTGTCTCTA	ATAGCTGCTA	ATGCAGAAAT	CGTATCGGCA	TCTTGACGTG
	3651	TTTGGTAACA	AACATTTTGA	ATCCAAACAT	GTTTTCTACC	TTGAATCGAA
	3701	ATACTAAGGT	GGTCCATATA	TGTGTCATTA	TGGGTTTCAA	ACACTTGTAC
45	3751	AATATCACGA	TGTTGATCAC	CGACTTTTTT	AAAAATGATA	ATCATATTGT
	3801	TAGAAAATCG	TTTTAATAAT	TCAAACGACA	CATGTTCTAA	TGCATTCAAT
	3851	TTTATTTCCG	ATGATAGTTG	ATACAAATCT	TTAATCCCAT	CATTATTTTT
	3901	AGCTAGAACA	ACTGTTTCGA	CTGTATTTAA	TCCATTTGTC	ACATATATTG
	3951	TCATACCAAA	AATCGGTTTA	ATGTTATTTG	CTATACATGC	ATCATAAAAT
50	4001	TTAGGAAAAC	CATACAATAC	ATTGGTGTCA	GTTATGGCAA	GTGCATCAAC
	4051	ATTTTCAGAC	ACAGCAAGTC	TTACGGCATC	TTCTATTTTT	AAGCTTGAAT

5 4101 TTAACAAATC ATAAGCCGTA TGAATATTTA AATATGCCAC CATGATTGAA
 4151 TGGCCCCCTT CTATTAGTTA AGTTTTGTGC GTAAAGCTGT AGCAAGTTGC
 4201 TCAAATTCAT CCCAGCTGTC CAACTGAAAY TCCTGACGCA TTCGGATGAC
 4251 CACCGCCACC AAAATCTTGC GCAATATCAT TAATAATCAA TTGCCCTTTA
 4301 GAACGTAATC GACATCTGAT TTCATTACCT TCATCGACTG CAAATACCCA
 4351 TATTTTCAAG CCTTTGATGT CAGCAATTGT ATTAACAAAC TGAGATGCTT
 4401 CATTTGGCTG AATACCGAAT TGCTCCAATA CATCTTCAGT TATTTTAACT
 4451 KGGCAGAATC CATCATCCAT AAGTTCGAAA TGTTGYAAAA CATAACCTTG
 4501 AAACGGCAAC ATTKYTGGGT CCTTCTCCAT CATTTTATTT AAAAGCGCAT
 10 4551 TATGATCAAT ATCATGCCCA ATTAACCTTC CAGCAATTTT CATAGTATGT
 4601 TCWGAGGTAT TGTTAAAAAG GRGATCGCCC AGTATCACCG ACGATACCAA
 4651 GATATAAAAC GCTCGCGATA TCTTTATTAA CAATTGCTTC ATCATTAATA
 4701 TGTGAGATTA AATCGTAAAT GATTTCACTT GTAGATGACG CGTTCGTATT
 4751 AACTAAATTA ATATCACCAT ACTGATCAAC TGCAGGATGA TGATCTATTT
 15 4801 TAATAAGTYT ACGACCTGTA CTATAACGTT CATCGTCAAT TCGTGGAGCA
 4851 TTGGCAGTAT CACATACAAT TACAAGCGCA TCTTGATATG TTTTATCATC
 4901 AATGTTATCT AACTCTCCAA TAAACTTTAA TGATGATTCC GCTTCACCCA
 4951 CTGCAAATAC TTGCTTTTGC GGAAATTTCT GCTGAATATA GTATTTTAAA
 5001 CCAAGTTGTG AACCATATGC ATCAGGATCK RSTYTARMRK RTCYSYGKMT
 20 5051 AMYRATTGYA TCGTTGTCTT CGATACATTT CATAATTTCA TTCAAAGTAC
 5101 TAATCATTTT CAWACTCCCT TTTTGTAGAA AGTGGCTTAA TTTAAGCATT
 5151 AGTCTATATC AAAATATCTA AATTATAAAA ATTGTTACTA CCATATTAATA
 5201 CTATTTGCCC GTTTTAATTA TTTAGATATA TATATTTTCA TACTATTTAG
 5251 TTCAGGGGCC CCAACACAGA GAAATTGGAC CCCTAATTTT TACAAACAAT
 25 5301 GCAAGTTGGG GTGGGGCCCC AACGTTTGTG CGAAATCTAT CTTATGCCTA
 5351 TTTTCTCTGC TAAGTTCCTA TACTTCGTCA AACATTTGGC ATATCACGAG
 5401 AGCGCTCGCT ACTTTGTCGT TTTGACTATG CATGTTCACT TCTATTTTGG
 5451 CGAAGTTTCT TCCGACGTCT AGTATGCCAA AGCGCACTGT TATATGTGAT
 5501 TCAATAGGTA CTGTTTAAAT ATACACGATA TTAAAGTTCT CTATCATGAC
 30 5551 ATTACCTTTT TTAAATTTAC GCATTTTATA TTGTATTGTT TCTTCTATAA
 5601 TACTTACAAA TGCCGCTTTA CTTACTGTTC CGTAATGATT GATTAAAGT
 5651 GGTGAAACTT CTACTGTAAT TCCATCTTGA TTCATTGTGA TATATTTGGC
 5701 GATTTGATCC TCTAGAGT

35

Mutant: NT78

Phenotype: temperature sensitivity

40 **Sequence map:** Mutant NT78 is complemented by pMP115, which contains a 5.3 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 51, along with open boxes to indicate the percentage of the clone for which DNA sequence has been obtained. Database searches at
 45 both the nucleic acid and peptide levels reveal no significant similarities between the sequences obtained at the left-most and right-most edges and any published sequences. The sequence generated from a Msp I subclone,

however, matches at both the nucleic acid and peptide level to *hsp60*, encoding the GroEL protein from *S. aureus* (Genbank Accession No. D14711). The relative size and orientation of the GroEL ORF is depicted by an arrow;
 5 other proteins (i.e. GroES) are known to reside near the identified ORF and will be confirmed by further DNA sequencing.

DNA sequence data: The following DNA sequence data represents the sequence generated by sequencing the left-most and rightmost edges of pMP115 and its subclone 78.3, starting with standard M13 forward and M13 reverse sequencing primers. The sequence below can be used to design PCR primers for the purpose of amplification from
 15 genomic DNA with subsequent DNA sequencing.

clone pMP115, a 5,300 bp genomic fragment

SEQ ID NO. 49

20 pMP115.m13f Length: 513 nt
 1 TTCTTGCCTC CCAATCGCCT AATAGCCCTN AAACTACTT TTTTAACTCT
 51 ATAGGCGATG TAAAAATACC ATATATTGAN GGTGCTATAC CTCCTAAAAT
 101 AGCAGTTCCC AAAGTTGTCA TTAGTGAAAT TACTGCGAAA GTATCATCCG
 151 AAAGCAATAA ATTCAACTA ATGCATTGTT TATTACCCAT CGAATTTATT
 25 201 GACCAAATAG CTAGAGAAAT AAACAACCCA AAATTTAAAA TAAATGATAT
 251 AGTAATAGCA ATTGTTTACA AACACGGAA TTTTTCATTT TTATTTATAT
 301 TATCCATTTT NCTCCCTTTT NCTTAAATCA TTTTATTATA TATTNCAATA
 351 ATCAATCTGA AATGTTGATG TAATTTGNN AATATATCAT ACTTTNCTC
 401 CTGAAAACCT CCCTAAATCA TCAATATGGN AATCNGTNTT NGGGTATTGC
 30 451 GNTTNCAACT CTTTAAANC TCACTCNTTC TTCTCATCGN CTTAACCGTA
 501 CTATCANTAA AAT

SEQ ID NO. 50

35 pMP115.m13r Length: 533 nt
 1 CTGAGCTGCT TNCANNNCCA NTNTGAAAAA GCCCCCAGNN CAGCCCAGNTT
 51 NCAAAACAAC GNCTNCATTT GAANCCCCAT GAAAAAGAAC GAATTTTGAC
 101 AATGGNTTAA AAAACANGNA AGATAATAAG AAAAAGTGCC GTCAACTGCA
 151 TATAGTAAAA GTTGGCTAGC AATTGTATGT NCTATGATGG TGGTATTTTC
 201 AATCATGCTA TTCTTATTTG TAAAGCGAAA TAAAAAGAAA AATAAAAACG
 40 251 AATCACAGCG ACGNTAATCC GTGTGTGAAT TCGTTTTTTT TATTATGGAA
 301 TAAAAATGTG ATATATAAAA TTCGCTTGTC CCGTGGCTTT TTTCAAAGCC
 351 TCAGGNTTAA GTAATTGGAA TATAACGNCA AATCCGTTTT GTAACATATG
 401 GGTAATAATT GGGAACAGCA AGCCGTTTTG TCCAAACCAT ATGCTAATGN
 451 AAAAATGNCA CCCATACCAA AATAAACTGG GATAAATTTG GNATCCATTA
 45 501 TGTGCCTAAT GCAAATNCCT NATGACCTTC CTT

The following DNA sequence data were acquired using standard sequencing methods and the commercially-available T7 and SP6 primers and can be used to demonstrate identity to the GroEL protein from *S. aureus*:

5

subclone 78.3, a 2000 bp Msp I fragment

SEQ ID NO. 51

78.3.sp6 Length: 568 nt

```

10      1  CCGACAGTCG TTCCCNATCAT GCAAAATATG GGGGCTAAAC TCAGTTCAAG
      51  AAGTCGGCAA ATAAGACAAA TGAAATTGCC TGGTGACGGT AGNACAACCTG
     101  CAACAGTATT AGCTCAAGCA ATGATTCAAG AAGGCTTGAA AAATGTTACA
     151  AGTGGTGCAG ACCCAGTTGG TTTACGACAA GGTATCGACA AAGCAGTTAA
     201  AGTTGCTGTT GAAGCGTTAC ATGAAAATTC TCAAAAAGTT GAAAATAAAA
     15  251  ATGAAATTNC GCAAGTAGGT GCGNTTTCAG CAGCAGATGN AGNAATTNGA
     301  CGTTATATTT CTGAAGCTAT NGGNAAGTA GGTAACGNTG GTGTCATTAC
     351  ANTTNTNGGG TCAAATGGGC TNTNCACTNN NCTNGANGTG GTTGNNGGTG
     401  TNCNATTTGA TCNNNGTTAT CANTCACCNN CTATNGTTAC TGCTTCNGCT
     451  AAAATGGTTG CTGCNTTTGG NCGCCCTAC ATTTTGTNA CNGCTTNGGG
     20  501  ANTCTCGTCT TTNCNCGATT CTTTCCCCTT TTTGGCCCT GGGNAATCTT
     551  TTNGGNCNCC CTTTATTT
  
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25

Mutant: NT81

Phenotype: temperature sensitivity

Sequence map: Mutant NT81 is complemented by clone 81-3, which contains a 1.7 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 52, along with open boxes to indicate the percentage of the clone for which DNA sequence has been obtained.. Database searches at both the nucleic acid and peptide levels reveal identity to the *fib* locus, encoding a fibrinogen binding protein, from *S. aureus* (Genbank Accession No. X72013; published in Boden, M.K. et al., *Mol. Microbiol.* 12 (1994) 599-606.) The relative size and orientation of the *Fib* ORF with respect to the restriction map is depicted by an arrow; also identified in this analysis is an ORF of unknown function downstream from (3' to) the *Fib* ORF.

DNA sequence data: The following DNA sequence data represent the sequences at the left-most and right-most edges of subclones pMP1043 and pMP1042, using standard SP6 and T7 sequencing primers. The sequences below can be used

45

to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

subclone 1042, a 400 bp Hind III fragment

5

SEQ ID NO. 52

1042.con Length: 437 nt

```

      1  CAAYTTAGYC AACTACTACC AATATAGCAC TAGAACTGGA AATGATAATT
     51  TAATATTGKG CACTTTTSA TTGKTAAAC ATGTACATAT TTNAAAAAAT
    101  AGGAGAGCAA AGKAAATAAT TGATATAGTT ATTTTSAGAG TAATCCTAGG
    151  AACTATTGTA TTTATATTS TCTCCCCTAC TTTTAAATGT CATTCAATTAT
    201  ACATAAGCAT TTTGATATAG AATTTATCAC ATATGCAAAT TGAAAACAGG
    251  TTAAGACCAT TTTTGTCTC AACCTGTTTT ATTTATTATC TATTTMTAAT
    301  TTCATCAATT TCTTGTATA TTTTCTYCTA TGCAACTTTA GCATCAGCCA
    351  TTGATACGAA ATCATTTTTC TTAAGTGCCG CTTTAGCTCT ATATTCATTC
    401  ATYATAATCG TACGTTTATA ATATGGATTT ACGTTGA
  
```

subclone 1043, a 1300 bp EcoR I/ Hind III fragment

20 SEQ ID NO. 53

1043.t7 Length: 659 nt

```

      1  CCCGATTGCA GCTCGGTACC GGNGATCCTC TAGAGTCGAT CTATCAAGCA
     51  GTAAATGAAA AAATGGACAT TAATGATATT AATATCGACA ATTTCCAATC
    101  TGTCTTTTTT GACGTGTCTA ATTTGAATTT AGTAATTCTA CCAACGTAA
    251  TCATTAGCTG GGTCACAATA TTTAACTATA GAATGAGAAG TTACAAATAA
    201  AATCTATGAG ATTATACCTN CAGACACCAA CATTCAAATG GTGTCTTTTN
    251  TGTGTGTGG TTTTATTTNT GAAATNCGAA AAAGTAGAGG CATGAATTTT
    301  GTGACTAGTG TATAAGTGCT GATGAGTCAC AAGATAGATA GCTATATTTT
    351  GTCTATATTA TAAAGTGTTT ATAGNTAATT AATAATTAGT TAATTTCAAA
    401  AGTTGTATAA ATAGGATAAC TTAATAAATG TAAGATAATA ATTTGGAGGA
    451  TAATTAACAT GAAAAATAAA TTGATAGCAA AATCTTNATT AACATTAGGG
    501  GCAATAGGTA TTAACAAC TACAATTGCG TCAACAGCAG ATGCGAGCGA
    551  AGGATACGGT CCAAGAGAAA AGAAACCAGT GAGTATTAAT CACAATATCG
    601  NAGAGTACAA TGATGGTACT TTTAATATCA ATCTTGANCA AAATTACTCA
    651  ACAACCTAA
  
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SEQ ID NO. 54

1043.sp6 Length: 298 nt

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      1  AATNCTCCTC CNATGNTTTA TNATGAAACT AACTTTAAGT NAAATATTTN
     51  TCCAGACTAC TTGCATCTCC NTTATNCCCT TCTATAGTTN CTATCCCAGT
    101  TNATGATAAA AGTAATGCTA ATGTNCCTGT NAATATATAT TTNTAAAATT
    151  NNATTATAAG CNCTCCTTAA AATTNATACT TACTGAGTAT ATAGTCAATT
    201  TNNGGACAAT TACATTAACC TGTCATTAAA TNGATTACTT TTTNNATTAA
    251  CAAAAATTAA CATAACATTT AATTAATTNT TTCCNGATAN CAGCAACG
  
```

Mutant: NT86

Phenotype: temperature sensitivity

Sequence map: Mutant NT86 is complemented by pMP121, which contains a 3.4 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 53, along with open boxes to indicate the percentage of the clone for which DNA sequence has been obtained.. Database searches at both the nucleic acid and peptide levels reveal identity at the nucleic acid and peptide levels to the *dnaK/dnaJ* genes, encoding Hsp70 and Hsp40, from *S. aureus* (Genbank Accession No. D30690; published in Ohta, T. et al. *J. Bacteriol.* 176 (1994) 4779-4783). Cross complementation studies (plasmid pMP120; data not shown) reveal that the ORF responsible for restoring a wild-type phenotype to mutant NT86 codes for Hsp40. The relative sizes and orientations of the identified genes are depicted in the restriction map by arrows.

DNA sequence data: The following DNA sequence data represent the sequences at the left-most and right-most edges of clone pM121, using standard M13 forward and M13 reverse sequencing primers. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP121, a 3400 bp genomic fragment

SEQ ID NO. 55

pMP121.m13f Length: 535 nt

```

1  TCCAAATATT CACCAAGCTG TAGTTC AAGA TGATAACCCT NATTTTAAANT
51 CTGGCGAAAT CACTCAAGAN CTACAAAAG GATACAAGCT TAAAGATAGA
101 GTATTAAGAC CATCANTGGT CAAAGTAAAC CAATAACTTA AATTGGCGA
151 AAAGACATTG TTAAAATTA ANTAAATTTA ATGATTAATT GGAGGNATTT
35 201 TTTTATGAGT AAAATTNTTG GTATAGACTT AGGTACAACA NATTCATGTG
251 TAACAGTATT AGANGGCGAT GAGCCAAAAG TAATTCAAAA CCCTGANGGT
301 TCACGTACAA CACCATCTGT NGTAGCTTTC AAAAATGGAG AAACCAAGT
351 TGGTGAAGTA GCAAAACGTC AAGCTATTAC AAACCCAAAC ACTGTTTCANT
401 CTATTAGNCG TCATATGGGT ACTGNTTATA ANGTAATAT TGAGGGTAAA
40 451 TCATACACAC CACAAGNNNT CTCAGCTNTG NTTTTNCAA ACTTANNANT
501 TNCAGCTGNA GTNATTTAGG TGNGNNGTT GNCAA

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SEQ ID NO. 56

pMP121.m13r Length: 540 nt

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45 1  ATGACTGCAG GTCGATCCAT GATTTACAAG TATATTGGTA GCCAATTCTA

```

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51 CTGCTTCATG ATTAATAATA ATTGAAAGCT CTGTCCAGTT CATACTTTAT
101 TCTCCCTTAA AGAATCTTTT TGNTCTATCT TTAAAATTCG AAGGTTGTTC
151 ATTAATTTCT TCACCATTTA ATTGGGCAAA TTCTTTCATT AGTTCTTTNT
201 GTCTATCTGT TAATTTAGTA GGC GTTACTA CTTTAATATC AACATATAAA
5 251 TCTCCGTATC CATAGCCATG AACATTTTTT ATACCCTTTT CTTTTAAGCG
301 GAATTGCTTA CCTGTTTGTG TACCAGCAGG GGATTGTTAA CATAACTTCA
351 TTATTTAATG TTGGTATTTT TATTTTCATCG CCTAAAGCTG CTTGTGGGAA
401 GCTAACATTT AATTTGNAAT AAATATCATC ACCATCACGT TTAAATGTTT
451 CAGATGGTTT AACTCTAAAT ACTACGTATT AATCANCAGG AGGTCCTCCA
10 501 TTCACGGCTG GAGAGGCTTC AACAGCTAAT CTTATTTGGT

```

The following DNA sequence data were acquired using standard sequencing methods and the commercially-available T7 and SP6 primers and can be used to demonstrate identity to the Hsp40 protein from *S. aureus*.

subclone 1116, a 1400 bp EcoR I/ Hind III fragment

SEQ ID NO. 57

```

20 1116.sp6 Length: 536 nt
    1 TTTATAATTT CATCTNTTGA AGCATCCTTA CTAATGCCTA AAACCTTCATA
    51 ATAATCTCTT TTGGCCACAG CTATCTCTCC TTINCTNAAT TAACATCATAT
101 AGTTTAAACGT AATATGTCAT ACTATCCAAA TAAAAAGCCA AAGCCAATGT
151 NCTATTGACT TTNACTTTTC ANATCATGAC AACATTCTAA TTGTATTGTT
25 201 TAATTATTTT NTGTCGTCGT CTTTINACTTC TTTAAATTCA GCATCTTCTA
251 CAGTACTATC ATTGTTTNA CCAGCATTAG CACCTTGINT TGTTGTTGCT
301 GTTGAGCCGC TTGCTCATAT ACTTTTINCTG NTAATTCTTG ANTCACTTTT
351 TCAAGTTCTT CTTTTTTAGA TTTANTATCT TCTATATNCT TGACCTTTCT
401 AANGCAGTTT TAAGAGCGTC TTTTTTCCTC TTTCTGCAGT TTTNTTATAC
30 451 TTCCTTTCAC CGTNATTTTT CGGCTTATTT CAGTTAAANG TTTTCCANC
501 TTGGGTNTAN CTATGGCTAG NAAAGNTTCG NTTCTT

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SEQ ID NO. 58

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    1116.t7 LENGTH: 537 nt
35 1 AAGATAAAAT GGCATTACAA CGTTTNAAG ATGCTGCTGA AAAANCTAAA
    51 AAAGACTTAT CAGGTGTATC ACAAACCTCA ATCTCATTAC CATTTATCTC
101 AGCTGGTGAA AACGGTCCAT TACACTTAGA AGTAAACTTA ACTCGTNCTA
151 AATTTGAAGA ATTATCAGAT TCATTAATTA GAAGANCAAT GGAACCTACA
201 CGCCAAGCAA TGAAAGACGC TGGCTTAACA AACTCAGATA TCGATGAAGT
40 251 TATCTTAGTT GGTGGNTCAA CTCGTATTCC AGCAGTACAA GANGCTGTCA
301 AAAAAAGAAAT CGGTAAAGAG CCTAACAAAG GAGTAAACCC GGNCGAAGTA
351 GGTGGCAATG GGNGCTGCAA TCCAAGGTGG CGTTATTCAC AGGTGACGTT
401 TAAAGACGTG TATTATTAGG NCGTAACACC ACTATCTTTA GGTATTGAAA
451 TTTTAGGTGG NCGTATGNAT TACGGTAATT GAACGTAACA CTACGGTTCC
45 501 TNCATTCTAA NTCTCAAAAT CTNTTCAACA GCAGTT

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Mutant: NT89

Phenotype: temperature sensitivity

Sequence map: Mutant NT89 is complemented by pMP122, which
 5 contains a 0.9 kb insert of *S. aureus* genomic DNA. A
 partial restriction map is depicted Fig. 54, along with
 open boxes to indicate the percentage of the clone for
 which DNA sequence has been obtained. Database searches at
 both the nucleic acid and peptide levels reveal a high
 10 level of similarity at the peptide level to the *trmD* gene,
 encoding (guanine-N1-) methyltransferase (EC 2.1.1.31),
 from various prokaryotes, including *S. marcescens* (Genbank
 Accession No. L23334; published in Jin, S. et al. Gene 1
 (1994) 147-148), *H. influenzae*, *E. coli*, and *S.*
 15 *typhimurium*. The predicted size and relative orientation
 of the *TrmD* ORF is depicted by an arrow.

DNA sequence data: The following DNA sequence data
 represent the sequences at the left-most and right-most
 20 edges of clone pM122, using standard M13 forward and M13
 reverse sequencing primers. The sequence below can be used
 to design PCR primers for the purpose of amplification from
 genomic DNA with subsequent DNA sequencing; it can also be
 used to demonstrate similarity to the *trmD* gene of *S.*
 25 *marcescens*:

clone pMP122, a 925 bp genomic fragment

SEQ ID NO. 59

30 pMP122.con Length: 925 nt

1	CTAGAGTCGA	TCTAAAGAAT	ATNTAANTCC	TNATATKSCT	GATGTTGTAA
51	AAGAAGTGGA	TGTTGAAAAT	AAAAAAATTA	TCATCACGCC	AATGGAAGGA
101	TTGTTGGATT	AATGAAAATT	GATTATTTAA	CTTTATTTCC	TGAAATGTTT
151	GATGGTGTTT	TAAATCATTC	AATTATGAAA	CGTGCCANG	AAAACAATAA
35 201	ATTACAAATC	AATACGGTTA	ATTTTAGAGA	TTATGCAATT	AACAAGCACA
251	ACCAAGTAGA	TGATTATCCG	TATGGTGGCG	GWCAAGGTAT	GGTGTTAAAG
301	CCTGACCCTG	TTTTTAATGC	GATGGAAGAC	TTAGATGTCA	CAGAMCAAAC
351	ACGCGTTATT	TTAATGTGTC	CACAAGGCCA	GCCATTTTCA	CATCAGAAAG
401	CTGTTGATTT	AAGCAAGGCC	GACCACATCG	TTTTCATATG	CGGACATTAT
40 451	GAAGGTTACG	ATGAACGTAT	CCGAACACAT	CTTGTCACAG	RTGAAATATC
501	AATGGGTGAC	TATGTTTTAA	CTGGTGGAGA	ATTGCCAGCG	ATGACCATGA
551	CTGATGCTAT	TGTTAGACTG	ATTCCAGGTG	TTTTAGGTAA	TGNACAGTCA
601	CATCAAGACG	ATTCATTTTC	AGATGGGTTA	TTAGAGTTTC	CGCAATATAC
651	ACGTCCGCGT	GAATTTAAGG	GTCTAACAGT	TCCAGATGTT	TTATTGTCTG
45 701	GAAATCATGC	CAATATTGAT	GCATGGAGAC	ATGAGCAAAA	GTTGAACCGC

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751 ACATATAATN AAAGACCTGA CTTAATTNNA AAATACCCAT TAANCCAATG
801 GCAGCATAAG GCAAATCATT CAGNAAANAT CATTAAAATC AGGTATTNGT
851 AAAAAGGTTN AGTGATTGTG NNNAACNNAN TNGNATGTGG CAAACATNCN
901 AANTACATCC TGGAAGGACC TCACG

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5

Mutant: NT94

10 **Phenotype:** temperature sensitivity

Sequence map: Mutant NT94 is complemented by pMP170, which contains a 2.5 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 55. Database searches at both the nucleic acid and peptide levels reveal strong peptide-level similarities to *yabM*, a hypothetical ORF of uncharacterized function from *B. subtilis*, noted as being similar to the *spoVB* gene from *B. subtilis*; further similarities are noted to hypothetical ORFs from *E. coli* and *H. influenzae*.

20 **DNA sequence data:** The following DNA sequence data represents the sequence generated by primer walking through clone pMP170, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP170

30

SEQ ID NO. 60

pMP170 Length: 2531 nt

```

35      1 TGGYTTTRTTT CAACATAATA TAGACATTTY CAATGTTATT CTATTAATTC
      51 TCCACGAAAC TGTTATCTTA TCGTTTTCTG GTTCTAATAT GTGTTTTTTG
     101 GGTGATTTAA TTA CTGTTTGC CGTTGAACAT TTACAAGGCC TTTTTTAAGT
     151 TAACTGTTTG ACCTCATTAC GTGTACCGAC GCCCATATTT GCTAAAAAAT
     201 TATCTATTCT CATCGTAAAA ACCTAACTCT ACGTCTTAAT TTTTCAGGAA
     251 TTTACCTTAA GAATTCGTCC GCAAGACGCG TTTTAATTGT GAWTGTACCG
40     301 TAAATTAGAA TACCTACTGT AACACCTAAA ATAATAATGA TTAAGTWACC
     351 AAGTTTTAGT AGGTYCTAAR AATARATTTG CAAGGNAAAA TACTAATTCT
     401 ACACCTAGCA TCATAATNNT GNATACAAGG ATATWTWTGC AAAATGGATC
     451 CCAACTATAG CTGAATTTAA ACTTCGCATA TWTTTTAAGR ATWTAGRAAT
     501 TACATCCMAT TGCAAATAAT TAATGCGATA CTAGTACGTA AAATTGCACC
45     551 AGGTGTATGG AATAACATAA TTAATGGATA GTTTAACGCT AACTTGATAA
     601 CTACAGAAGC TAAAATAACA TAAACTGTTA ATTTCTGTTT ATCTATACCT

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5 651 TGTAANATNG ATGCCGTTAC ACTTAATAGT GAAATYAGTA TTGCTACAGG
 701 CGCATAATAK AATAATAAGC GACTACCATC ATGGTTAGGG TCATGACCTA
 751 WAACAATTGG ATCGTAACCA TAGATAAACT GTGAAATTAA TGGTTGTGCC
 801 AAGGCCATAA TCYCCAATAC TAGCTGGGAA CAGTTATAAA CATTWAGTTA
 851 CACCAATTAG ATGTTCTTAA TTTGATGATG CATTTCATGT AAGCGACCTT
 901 CTGCAAATGT TTTTGTAATA TAAGGAATTA AACTCACTGC AAAACCAGCA
 951 CTTAATGATG TCGGAATCAT TACAATTTTA TTAGTTGACA TATTTAGCAT
 1001 ATTAAAGAAT ATATCTTGTA ACTGTGAAGG TATACCAACT AAAGATAAAG
 1051 CACCGTTATG TGTAAATTGA TCTACTAAGT TAAATAATGG ATAATTCAAA
 1101 CTTACAATAA CGAACGGTGA TACTATAAGC AATAATTTCT TTATACATCT
 1151 TGCCATATGA CACATCTATA TCTGTGTAAT CAGATTCGAC CATACGATCA
 1201 ATATTATGCT TACGCTTTCT CCAGTAATAC CAGAGTGTGR ATATRCCAAT
 1251 AATCGCACCA ACTGCTGCTG CAAAAGTAGC AATACCATTG GCTAATAAAA
 1301 TAGAGCCATC AAAGACATTT AGTACTAAAT AACTTCCGAT TAATATGAAA
 1351 ATCACGCGTG CAATTTGCTC AGTTACTTCT GACACTGCTG TTGGCCCCAT
 1401 AGATTTATAA CCTTGGAATA TCCCTCTCCA TGTCGCTAAT ACAGGAATAA
 1451 AGATAACAAC CATACTAATG ATTCTTATAA TCCAAGTTAA TATCATCCGA
 1501 CTGACCAACC GTTTTTATCA TGAATGTTTC TAGCTAATGT TAATTCAGAA
 1551 ATATAAGGTG YTAAGAAATA CAGTACCAAG AAACCTAAAA CACCGGTAAT
 20 1601 ACTCATTACA ATAAAAAYTCG ATTTATAAAA WTTCTGACTT WACTTTAWAT
 1651 GCCCCAATAG CATTATATTT CGCAACATAT TTCGAAGCTG CTAATGGTAC
 1701 ACCTGCTGTC GCCAAGTCA ATTGCAATAT TATATGGTGC ATAAGCGTWT
 1751 GTTGAACGGS GCCATATTTT CTTGTCCNC CAATTAAATA GTTGAATGGA
 1801 ATGATAAAAA GTACGCCCAA TACCTTGGTA ATTAATATAC TAATGGTAAT
 25 1851 TAAAAAGGTT CCACGCACCA TTTCTTTACT TTCACTCATT ACGAATCTCC
 1901 CTATCTCATG TTTATTAAAG TTTTGTAAC TAAAAGCTGT TTCTCTGTAA
 1951 AATCATTTTT CATTATTATG AATATATCAC AAAACTTTAT TTCATYGTG
 2001 TATATTTCAA TGGAATTATC CATAACAAAA TTATCAACAC ATTGTCATTG
 2051 AATACTAGAT TTTGATTAGA ATATTACGAA ATTTATATA AACATTATAC
 30 2101 TACTATTTGA GATGAACATC GCATAACAGT AGAAAAATCA TTCTTATCAT
 2151 ACACATACAT CTTCAATTTT TATGAAGTTC ACATTATAAA TATATTCAAC
 2201 ATAATTGTCA TCTCATAACA CAAGAGATAT AGCAAAGTTT AAAAAAGTAC
 2251 TATAAAATAG CAATTGAATG TCCAGTAACA AATTTGGAGG AAGCGTATAT
 2301 GTATCAAACA ATTATTATCG GAGGCGGACC TAGCGGCTTA ATGGCGGCAG
 35 2351 TAGCWGCAAG CGAACAAAGT AGCAGTGTGT TACTCATTGA AAAAAAGAAA
 2401 GGTCTAGGTC GTAAACTCAA AATATCTGGT GGCGGTAGAT GTAACGTAAC
 2451 TAATCGAYTA CCATATGCTG AAATTATTCA AGGAACATTC CCTGGAAATG
 2501 GGAAATTTY ATCATAGTTC CCTTTTCAAT T

40

Mutant: NT96

Phenotype: temperature sensitivity

45 **Sequence map:** Mutant NT96 is complemented by pMP125, which contains a 2.6 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 56, along with open boxes to indicate the percentage of the clone for which DNA sequence has been obtained. Database searches at both the nucleic acid and peptide levels reveal strong

similarities at the peptide level to the *murC* gene product, encoding UDP-N-Acetyl muramoyl-L-alanine synthase (EC 6.3.2.8), from *B. subtilis* (Genbank Accession No. L31845).

- 5 **DNA sequence data:** The following DNA sequence data represent the sequences at the left-most and right-most edges of clone pM125, using standard M13 forward and M13 reverse sequencing primers. The sequences below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing:

clone pMP125

SEQ ID NO. 61

15 pMP125.forward Length: 889 nt

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      1 TCGAGCTCGG TACCCGGGGA TCCTCTAGAG TCGATCTACA GAGCTGTTTA
     51 ACGTTTGTAC TGAGTCACCG ATACCTTTAA CAGCATCTAC AACTGAGTTT
    101 AAACGATCTA CTTTACCTTG GATATCCTCA GTTAAACGGT TTACTTTATG
    20 151 AAGTAAATCT GTTGTTTCAC GAGTAATACC TTGAACTTGA CCTTCTACAC
     201 CGTCAAGTGT TTTTGCAACA TAATCTAAGT TTTTCTTAAC AGAATTTAAT
     251 ACAGCTACGA TACCGATACA TAAAATTAAG AATGCAATCG CAGCGATAAT
     301 TCCAGCAATT GGTAATAATCC AATCCATTAA AAACGCCTCC TAATTAACAT
     351 GTAATAATGT CATTAATAAT AAATACCCAT ACTACTCTAT TATAAACATA
    25 401 TTA AACGCA TTTTTCATGC CTAATTTATC TAAATATGCA TTTTGTAATT
     451 TTTGAATATC ACCTGCACCC ATAAATGAAA ATAACAGCAT TATCAAATTG
     501 TTCTAATACA TTAATAGAAT CTTCAATTAAT TAACGATGCA CCTTCAATTT
     551 TATCAATTAA ATCTTGWTWC GTTAATGCGC CAGTATTTTC TCTAATTGAT
     601 CCAAAAATTT CACAATAAGA AATACACGAT CTGCTTTACT TAACTTTTCT
    30 651 GCAAATTCAT TTA AAAATGC CTGTGTTCTA GAGAAAGTGT GTGGTTTGAN
     701 ATACTGCAAC AACTTCTTTA TGTGGATATT TCTTTCGTGC GGTTCATTT
     751 GNNGCACTAA NTTCTCTTGG ATGGTGTNCA TAATCAGCTA CATTAACTTG
     801 ATTTGCGATT GTAGTNTCAT NGANNGACGT TTAACNCCAC CAACGTTTCT
     851 AATGCTTCTT TAANATTGGG ACATCTAACT TCTCTAAA
  
```

SEQ ID NO. 62

pMP125.reverse Length: 902 nt

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      1 GCATGCCTGC AGGTGCATCC AAAAATGGTT GAATTAGCTC CTTATAATGG
    40 51 TTTGCCMMMT TTRGTTGCCA CCGKTAATTA CAGATGTCMA AGCCAGCTAC
    101 ACAGAGTTTG AAAAKGGSCC STWGAAAGGA AATGGAACGA ACGTKATAAG
    151 TTATTTGCCA CATTACCATG TACGTAATAT AACAGCCATT TAACAAAAAA
    201 GCCACCATAT GATGAAAGAW TGCCAAAAAT TGTCATTGTA ATTGATGAGT
    251 TGGCTGATTT AATGATGATG GCTCCGCAAG AAGTTGAACA GTCTATTGCT
    45 301 AGAATTGCTC AAAAAGCGAG AGCATGTGGT ATTCATATGT TAGTAGCTAC
     351 GCAAAGACCA TCTGTCAATG TAATTACAGG TTTAATTAAA GCCAACATAC
     401 CAACAAGAAT TGCATTTATG GTATCATCAA GTGTAGATTG GAGAACGATA
     451 TTAGACAGTG GTGGAGCAGA ACGCTTGTTA GGATATGGCG ATATGTTATA
  
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501 TCTTGGTAGC GGTATGAATA AACCGATTAG AGTTCAAGGT ACATTTGTTT
551 CTGATGACGA AATTGATGAT GTTGTTGATT TTATCAAACA ACAAAGAGAA
601 CCGGACTATC TATTTGAAGA AAAAAGAAAT TGTTGAAAAA AACACAAACA
651 CMATCMCMAG ATGAATTATT TGATGATGTT TGTGCATTTA TGGTTAATGA
701 AGGACATATT TCAACATCAT TAATCCAAAG ACATTTCCAA ATTGGCTATA
751 ATAGAGCAGC AAGAATTATC GATCAATTAG AAGCAACTCG GTTATGTTTC
801 GAGTGCTAAT NGGTTCAAAA ACCNAGGGAT GTTTATGTTA CGGAAGCCGA
851 TTTTAAATAA AGAATAATTT ATGATTAAGG ATTTTATAT AATGGACACC
901 CC

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Mutant: NT99

Phenotype: temperature sensitivity

Sequence map: Mutant NT99 is complemented by pMP176, which contains a 3.6 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 57. Database searches at both the nucleic acid and peptide levels reveal strong similarity at the peptide level to the *murG* gene, encoding UDP-GlcNAc:undecaprenyl-pyrophosphoryl-pentapeptide transferase, from *B. subtilis* (Genbank Accession No. D10602; published in Miyao, A. et al. *Gene* 118 (1992) 147-148.) Cross complementation studies (data not shown) have demonstrated that the minimal amount of clone pMP176 required for restoring a wild-type phenotype to mutant NT99 is contained in the right-half of the clone and contains the entire (predicted) *murG* ORF; the predicted size and orientation of this ORF is depicted in the restriction map by an arrow.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP176, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP176

SEQ ID NO. 63

pMP176 Length: 3592 nt

1 GATCCTTATT CTGAATATTT AACAAAWGCA ACAAACGAAA TCCCTTTGAA
 51 TGAAAGGTGT TTCAGGTGCA TTTTKTAGGT ATTGGTGCAG AAAATGCAAA
 101 AGAAAAATGA ATCAAATTAT GGTTACTAGT CCTATGAAGG GWTCTCCAGC
 151 AGAACGTGCT GGCATTTCGT CTAAAGATGT CATTACTAAA GTAAATGGAA
 5 201 AATCAATTAA AGGTAAAGCA TTAGATGAAG TTGTCAAAGA TGTTTCGTGGT
 251 AAAGAAAACA CTGAAGTCAC TTAACTGTT CAACGAGGTA GTGAAGAAAA
 301 AGACGTTAAG ATTAAACGTG RAAAAATTC TGTAAAAAGT GTTGAGTATW
 351 AGRAAAAAGG TAAAGTTGGA GTTATTACTA TTAATAAATT CCAGAMTGAT
 401 ACATCCAGGT GRATTGAAAG ATGCAGTTCT AAAAGCTCAC CAAAGATGGT
 10 451 TTGWAAAAGA TTGTTTTAGA TTTAAGAAAT AATCCAGGTG GACTACTAGA
 501 TGAAGCTGTT AAAATGGCAA ATATTTTTAT CGATAAAGGA AAAACTGTTG
 551 TTAACTARA AAAAGGTAAA GATACTGAAG CAATTCNNAC TTCTAATGAT
 601 GCGTTAAAAG AAGCGAAAAGA CATGGATATA TCCATCTTAG TGAATGAAGG
 651 TTCNGCTNGC GCTTCTGAAG TGTTTACTGG TCGCGTAAAA GACTNTAATA
 15 701 AAGCTAAAGT TTATGGGTCA AAAACATTCT GCAAAGGTGT CGTACAACT
 751 ACAAGAGAGT TTAAGGGATG GTTCATTGTT AAAATATACT GAAATGGAAA
 801 TGGTTAACGC CAGATGGTCA TTATATTAC NGTACAAGGC ATNAAACCAG
 851 ACGTTACTNT TTGACACACC TGAAATANCA ATCTTTTAAA TGTCAATCCT
 901 AATACGANAA CATTTAAAGT TNGGAGACGA TGAATCTAAA ATATTAAAAC
 20 951 TATTAATAWT GGTTTATCAG CTTTAGGTTA TAAAGTTGAT AAATGGAATC
 1001 AACGCCAATT TGGATAAAGC TTTAGAAAAT CAAGTTAAAG CTTYCCAMCA
 1051 AGCGAATAAA CTTGAGGTAM YKGGKGAWTT TAATAAAGAA ACGAATAATA
 1101 AATTTACTGA GTTATTAGTT GAAAAAGCTA ATAAACATGA TGATGTTCTC
 1151 GATAAGTTGA TTAATATTTT AAAATAAGCG ATACACACTA CTAATAATTGT
 25 1201 ATTATTATTA TGTTAATGAC ACGCCTCCTA AATTTGCAA GATAGCAATT
 1251 TAGGAGGCGT GTTTATTTTT ATTGACGTCT AACTCTAAAA GATATAAATT
 1301 AGACATTTAC AAATGATGTA AATAACGCAA TTTCTATCAT CGCTGATAAC
 1351 AATTCATGGT TTAATATGCA ATGAGCATAT ACTTTTTAAA TAGTATTATT
 1401 CACTAGTTTT AACAATCAAT TAATTGGTAT ATGATACTTT TATTGGTTAT
 30 1451 TTTTATCCCA TAGTGTGATA AWTACTATTT TTCATTAYA ATAAAGGTTT
 1501 AAAGCATGTT AATAGTGTGT TAAGATTAAC ATGTACTGAA AAACATGTTT
 1551 WACAATAATG AATATAAGGA KTGACGTTAC ATGAWCCGTC CTAGGTAATA
 1601 TGTCMGAWTT AGATCAAATC TTAAATCTAG TAGAAGAAGC AAAAGAATTA
 1651 ATGAAAGAAC ACGACAACGA GCAATGGGAC GATCAGTACC CACTTTTAGA
 35 1701 ACATTTTGAA GAAGATATTG CTAAAGATTA TTTGTACGTA TTAGAGGAAA
 1751 ATGACAAAAT TTATGGCTTT ATTGTTGTCTG ACCAAGACCA AGCAGAAATGG
 1801 TATGATGACA TTGACTGGCC AGTAAATAGA GAAGGCGCCT TTGTTATTCA
 1851 TCGATTAACT GGTTCGAAAG AATATAAAGG AGCTGCTACA GAATTATTCA
 1901 ATTATGTTAT TGATGTAGTT AAAGCACGTG GTGCAGAAGT TATTTTAACG
 40 1951 GACACCTTTG CGTTAAACAA ACCTGCACAA GGTTTATTTG CCAAATTTGG
 2001 ATTTCATAAG GTCGGTGAAC AATTAATGGA ATATCCGCCM TATGATAAAG
 2051 GTGAACCATT TTATGCATAT TATAAAAATT TAAAAGAATA GAGGTAATAT
 2101 TAATGACGAA AATCGCATTT ACCGGAGGGG GAACAGTTGG ACACGTATCA
 2151 GTAAATTTWA RTTTAATTCC AACTGCATTA TCACAAGGTT ATGGARGCGC
 45 2201 TTTATATTGG TTCTAAAAAT GGTATTGAAA GAGAGAATGA TTGAWTCACC
 2251 AACTACCCRG AAATTAAGTA TTATCCTATT TCGGAGTGKT AAATTAAGAA
 2301 GATATATTTT TTTAGAAAAT GCCAAAGACG TATTTAAAGT ATTGAAAGGT
 2351 ATTCTTGATG CTCGTAAAGT TTTGAAAAAA GAAAAACCTG ATCTATTATT
 2401 TTCAAAAGGT GGATTTGTAT CTGTGCCTGT TGTTATTGCA GCCAAATCAT
 50 2451 TAAATATACC AACTATTATT CATGAATCTG ACTTAACACC AGGATTAGCG
 2501 AATAAGATAG CACTTAAATT TGCCAAGAAA ATATATACAA CATTGGAAGA

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5  2551 AACGCTAAAC TACTTACCTA AAGAGAAAGC TGATTTTATT GGAGCAACAA
    2601 TTCGAGAAGA TTTAAAAAAT GGTAAATGCAC ATAATGGTTA TCAATTAACA
    2651 GGCTTTTATG RAAATAAAAA AGTTTTACTC GTYATGGGTG GAAGCTTWGG
    2701 AAGTAAAAAA TTAAATAGCA TTATTCGCGA AAACCTAGAT GCATTTATTA
    2751 CAACAATATC AAGTGATACA TTAACTGGT AAAGGATTAA AAGATGCTCA
    2801 AGTTAAAAAA TCAGGATATA TACAATATGA ATTTGTTAAA GNGGATTTAA
    2851 CAGATTTATT AGCAATTACG GATACAGTAA TAAGTAGAGC TGGATCAAAT
    2901 GCGATTTATG GAGTTCTTAA CATTACGTNT ACCAATGTTA TTAGTACCAT
    2951 TAGGTTTAGA TCAATCCCGA GGCGACCAAA TTGACANTGC AAATCATTTT
10  3001 GCTGATAAAG GATATGCTAA AGCGATTGAT GAAGAACAAT TAACAGCACA
    3051 AATTTTATTA CAAGAACTAA ATGAAATGGA ACAGGAAAGA ACTCGAATTA
    3101 TCAATAATAT GAAATCGTAT GAACAAAGTT ATACGAAAGA AGCTTTATTT
    3151 GATAAGATGA TTAAAGACGC ATTGAATTAA TGGGGGGTAA TGCTTTATGA
    3201 GTCAATGGAA ACGTATCTCT TTGCTCATCG TTTTACATT GGTTTTTTGA
15  3251 ATTATCGCGT TTTTCCACGA ATCAAGACTT GGGAAATGGA TTGATAATGA
    3301 AGTTTATGAG TTTGTATATT CATCAGAGAG CTTTATTACG ACATCTATCA
    3351 TGCTTGGGGC TACTAAAGTA GGTGAAGTCT GGGCAATGTT ATGTATTTCA
    3401 TTAATTCTTG TGGCATATCT CATGTTAAAG CGCCACAAAA TTGAAGCATT
    3451 ATTTTTTGCA TTAACAATGG CATTATCTGG AATTTTGAAT CCAGCATTAA
20  3501 AAAATATATT CGATAGAGAA AGGACCTGAC ATTGCTGGCG TTTGAATTGG
    3551 ATGATTAACA GGRTTTAGTT TTCCTGAGCG GTCATGCTAT GG

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25

Mutant: NT102

Phenotype: temperature sensitivity

Sequence map: Mutant NT102 is complemented by pMP129, which contains a 2.5 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 58 (there are no apparent restriction sites for EcoR I, Hind III, Bam HI or Pst I). Database searches at both the nucleic acid and peptide levels reveal strong similarity to one hypothetical ORF of unknown function from *Synechocystis* spp.; another ORF with no apparent homolog on the current databases is also predicted to be contained in this clone. The predicted sizes and orientations of these two hypothetical ORFs is depicted in the map.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP129, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of

amplification from genomic DNA with subsequent DNA sequencing.

clone pMP129

5

SEQ ID NO. 64

pMP129 Length: 2573 nt

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1  ATTCGAGCTC GGTACCCGKG GATCCTSYAG AGTCGATCCG CTTGAAACGC
10  51  CAGGCACTGG TACTAGAGTT TTGGGTGGTC TTAGTTATAG AGAAAGCCAT
    101  TTTGCATTGG AATTACTGCA TCAATCACAT TTAATTTCCCT CAATGGATTT
    151  AGTTGAAGTA AATCCATTGA TTGACAGTAA TAATCATACT GCTGAACAAG
    201  CGGTTTCATT AGTTGGAACA TTTTGTGGTG AAACTTTATT ATAAATAAAT
    251  GATTTGTAGT GTATAAAGTA TATTTTGCTT TTTGCACTAC TTTTTTTAAT
15  301  TCACTAAAAT GATTAAGAGT AGTTATAATC TTTAAAATAA TTTTTTCTA
    351  TTTAAATATA TGTTTCGTATG ACAGTGATGT AAATGATTGG TATAATGGGT
    401  ATTATGGAAA AATATTACCC GGAGGAGATG TTATGGATTT TTCCAACTTT
    451  TTTCAAAACC TCAGTACGTT AAAAATTGTA ACGAGTATCC TTGATTTACT
    501  GATAGTTTGG TATGTACTTT ATCTTCTCAT CACGGTCTTT AAGGGAACATA
20  551  AAGCGATACA ATTACTTAAA GGGATATTAG TAATTGTTAT TGGTCAGCAG
    601  ATAATTTWGA TATTGAACTT GACTGCMACA TCTAAATTAT YCRAWWYCGT
    651  TATTCMATGG GGGGTATTAG CTTTAAANAGT AATATTCCAA CCAGAAATTA
    701  GACGTGCGTT AGAACAACCTT GGTANAGGTA GCTTTTTTAAA ACGCNATACT
    751  TCTAATACGT ATAGTAAAGA TGAAGAGAAA TTGATTCAAT CGGTTTCAAA
25  801  GGCTGTGCAA TATATGGCTA AAAGACGTAT AGGTGCATTA ATTGTCTTTG
    851  AAAAAGAAAC AGGTCTTCAA GATTATATTG AAACAGGTAT TGCCAATGGA
    901  TTCAAATATT TCGCAAGAAC TTTTAATTAA TGTCTTTATA CCTAACACAC
    951  CTTTACATGA TGGTGCAAKG ATTATTCAAG GCACGAARAT TGCAGCAGCA
30  1001 GCAAGTTATT TGCCATTGTC TGRWAGTCCT AAGATATCTA AAAGTTGGGT
    1051 ACAAGACATA GAGCTGCGGT TGGTATTTCA GAAGTTATCT GATGCATTTA
    1101 CCGTTATTGT ATCTGAAGAA ACTGGTGATA TTTTCGGTAAC ATTTGATGGA
    1151 AAATTACGAC GAGACATTTT AAACCGAAAT TTTTGAAGAA TTGCTTGCTG
    1201 AACATTGGTT TGGCACACGC TTTCAAAAGA AAGKKKTGAA ATAATATGCT
    1251 AGAAAKTAAA TGGGGCTTGA GATTTATTGC CTTTCTTTTT GGCATTGTTT
35  1301 TTCTTTTTAT CTGTAAACAA TGTTTTTGGA AATATTCTTT AAACACTGGT
    1351 AATTCTTGGT CAAAAGTCTA GTAAAACGGA TTCAAGATGT ACCCGTTGAA
    1401 ATTCTTTATA ACAACTAAAG ATTTGCATTT AACAAAAGCG CCTGAAACAG
    1451 TTAATGTGAC TATTTAGGGA CCACAATCAA AGATAATAAA AATTGAAAAT
    1501 CCAGAAGATT TAAGAGTAGT GATTGATTTA TCAAATGCTA AAGCTGGAAA
40  1551 ATATCAAGAA GAAGTATCAA GTTAAAGGGT TAGCTGATGA CATTCAATTAT
    1601 TCTGTAAAAC CTAAATTAGC AAATATTACG CTTGAAAACA AAGTAACTAA
    1651 AAAGATGACA GTTCAACCTG ATGTAAGTCA GAGTGATATT GATCCACTTT
    1701 ATAAATTAC AAAGCAAGAA GTTTCACCAC AAACAGTTAA AGTAACAGGT
    1751 GGAGAAGAAC AATTGAATGA TATCGCTTAT TTAAGGCCA CTTTTAAAAC
45  1801 TAATAAAAAG ATTAATGGTG ACACAAAAGA TGTGCGAGAA GTAACGGCTT
    1851 TTGATAAAAA ACTGAATAAA TTAAATGTAT CGATTCAACC TAATGAAGTG
    1901 AATTTACAAG TTAAAGTAGA GCCTTTTAGC AAAAAGGTTA AAGTAAATGT
    1951 TAAACAGAAA GGTAGTTTRS CAGATGATAA AGAGTTAAGT TCGATTGATT
    2001 TAGAAGATAA AGAAATTGAA TCTTCGGTAG TCGAGATGAC TTMCAAAATA
50  2051 TAAGCGAAGT TGATGCAGAA GTAGATTTAG ATGGTATTTT AGAATCAACT
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2101  GAAAAGACTG  TAAAAATCAA  TTTACCAGAA  CATGTCACTA  AAGCACAACC
2151  AAGTGAAACG  AAGGCTTATA  TAAATGTAAA  ATAAATAGCT  AAATTAAAGG
2201  AGAGTAAACA  ATGGGAAAAT  ATTTTGGTAC  AGACGGAGTA  AGAGGTGTCTG
2251  CAAACCAAGA  ACTAACACCT  GAATTGGCAT  TTAAATTAGG  AAGATACGGT
5    2301  GGCTATGTTC  TAGCACATAA  TAAAGGTGAA  AAACACCCAC  GTGTACTTGT
2351  AGGTCGCGAT  ACTAGAGTTT  CAGGTGAAAT  GTTAGAATCA  GCATTAAATAG
2401  CTGGTTTGAT  TTCAATTGGT  GCAGAAGTGA  TGCGATTAGG  TATTATTTCa
2451  ACACCAGGTG  TTGCATATTT  AACACGCGAT  ATGGGTGCAG  AGTTAGGTGT
2501  AATGATTTCA  GCCTCTCATA  ATCCAGTTGC  AGATAATGGT  ATTAAATTCT
10   2551  TTGSCTCGAC  CNCCNNGCTN  GCA

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Mutant: NT114

15 **Phenotype:** temperature sensitivity

Sequence map: Mutant NT114 is complemented by pMP151, which contains a 3.0 kb insert of *S. aureus* genomic DNA. A partial restriction map is depicted Fig. 59. Database searches at both the nucleic acid and peptide levels reveal

20 strong similarity at the peptide level to the *dfp* gene, encoding a flavoprotein affecting pantothenate metabolism and DNA synthesis, from *E. coli* (Genbank Accession No. L10328; published in Lundberg, L.G. et al. *EMBO J.* 2 (1983) 967-971). The predicted size and orientation of the Dfp

25 ORF is represented by an arrow in the restriction map.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP151, starting with standard M13 forward and M13

30 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA

35 sequencing.

clone pMP151

SEQ ID NO. 65

pMP151 Length: 2976 nt

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40   1.  GRTCGACTCT  AGAGTCGATC  TTTAAATGGG  TCTCTTTCAA  CAACCGCGTC
      51  ATATTTTTMA  ACATAACCTT  TTTTRATAAG  TCCATCTAAA  CTGGATTTTR
     101  AAAAGCCCAT  ATCCTCAATA  TCAGTTAAAA  ATATTGTTTT  ATGTTGTTCT
     151  TCAGACAAGT  AAGCATACAA  ATCGTATTGT  TTAATAACTT  TCTCCAACCT
45   201  AGCTAATACT  TCATCAGGAT  GATACCCTTC  AATGACACGA  ACAGCACGCT
     251  TGGTTTTTTT  AGTTATATTT  TGTGTGAGAA  TCGTTTTTTC  TTCAACGATA

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301 TCATCTTTTA ACAACTTCAT AAGCAATTGA ATATCATTAT TTTTTTGCGC
351 ATCTTTTATA TAATAGTAAC CATGCTTATC AAATTTTTGT AATAAAGCTG
401 AAGGTAGCTC TATGTCATCT TTCATCTTAA ATGCTTTTTT ATACTTCGCT
451 TTAATAGCAC TCGGAAGCAT CACTTCTAGC ATAGAAATAC GTTTAATGAC
501 ATGAGTTGAA CCCATCCACT CACTTAAAGC TATTAATTCT GATGTTAATT
551 CTGGTTGTAT ATCTTTCAC TCTATGATTT TTTTAACTT CGAAACGTCA
601 AGTTGTGCAT CAGGTTCTGC TGTTACTTCC ATTACATAAC CTTGAATCGT
651 TCTTGGTCCA AAAGGTACAA TTACACGCAC ACCAGGTTGG ATGACAGATT
701 CGAGTTGTTC GGAATTATA TAATCAAATT TATAGTCAAC GCTCTTCGAC
751 GCGACATCGA CTATGACTTT CGCTATCATT ATKGCCACCT AGTTTCTAGT
801 TCATCTAAAA TTTGTGCAGC WAATACTACK TTTTKNCCTT YCTTGATATT
851 TACKTTTCA TTAKTTTTAA AATGCATTGT CAATTCATTA TCATCAGAAC
901 TAAATCCGAT AGACATATCC CCAACATTAT TTGAAATAAT CACATCTGCA
951 TTTTCTTGC GTAATTTTTG TTGTGCATAA TTTTCAATAT CTTCAGTCTC
1001 TGCTGCAAAG CCTATTAAAT ACTGTGATGT TTTATGTTCA CCTAAATATT
1051 TAAGAATGTC TTAGTACGT TTAAGATA CTGACAAATC ACCATCCTGC
1101 TTTTTCATCT TATGTTCTTA ATACATCAAC CGGTGTATAG TCAGATACGG
1151 CTGCTGCTTT TACAACAATA TYTTGTTCCG TYAAATCGGC TTGTCACCTG
1201 GTTCAAACAT TTCTTCAGGC ACTTTGRACA TGAATAACTT CAATATCTTT
1251 TGGATCCTCT AGTGTTGTAG GACCAGCAAC TAACGTCACG ATAGCTCCTC
1301 GATTTGCAA TGCTTCAGCT ATTGCATAGC CCATTTTTCC AGAAGAACGA
1351 TTGGATACAA ATCTGACTGG ATCGATAACT TCAATAGTTG GTCCTGCTGT
1401 AACCAATGCG CGTTTATCTT GAAATGAACT ATTAGCTAAA CGATTACTAT
1451 TTTGAAAATG AGCATCAATT ACAGAAACGA TTTGAAGCGG TTCTTCCATA
1501 CGTCCTTTAG CAACATAACC ACATGCTAGA AATCCGCTTC CTGGTTCGAT
1551 AAAATGATAC CCATCTTCTT TTAATAATT AATATTTTGC TGCGTTACGT
1601 TTATTTTCAT ACATATGCAC ATTCATAGCA GGCGCAATAA ATTTCCGGTGT
1651 CTCTGTTGCT AGCAACGTTG ATGTCACCAA ATCATCAGCA ATACCTACAC
1701 TCAATTTTGC AATTGTATTT GCCGTTGCAG GTGCAACAAT GATTGCATCK
1751 GCCCAATCCA CCTAATGCAA TATGCTGTAT TTCTGGAAGG ATTTTYYTCT
1801 ATAAAAGTAT CTGTATAAAC AGCATTTTCA MTTATTGCTT GAAATGCTAA
1851 TGGTGTCA CA AATTTTGTG CGTGATTCTG TAAACATAAC GCGAACTTCA
1901 TAACCCAGAT TGTGTTAACT TACTTGTCAT ATCAATTGCT TTATATGCCG
1951 CAATGCCACC TGTAACGGCT AATAATATTT TCTTCATATT CAATCTCCCT
2001 TAAATATCAC TATGACATTT ACGCTTTACA TCATCATATG CGCACAAATG
2051 CTCATTACTT TTTTATAGAT ACAAATTTAG TATTATTATA ACATCAATCA
2101 TTGGATAAAC TAAAAAACA CACCTACATA GGTGCGTTTG ATTTGGATAT
2151 GCCTTGACGT ATTTGATGTA ACGTCTAGCT TCACATATTT TTAATGGTCG
2201 AAACATTCTT TTACCATAAT AATCACTTGA AATAACAGGG CGAATTTTAC
2251 CGTCAGCAAT TTCTTCTAAC GCTCTACCAA CTGGTTTAAA TGAATGATAT
2301 TCACTTAATA ATTCAGTTTC AGGTTGTTCA TCAATTTTAC GCGCTCTTTT
2351 CGCTGCAGTT GTTGCAATTA AATACTTTGA TTTAATTTGT GACGTTAATT
2401 GGTTTAAAGG TGGATTTAAC ATTAATTTTT AGCCTCCAAA ATCATTTTTTC
2451 TATACTTAGC TTCTACGCGC TCTCTTTTTA AGTGCTCAGC TTCTACAATA
2501 CATTGAATTC TATTCTTCGC AAGTTCTACT TCATCATTAA CTACAACGTA
2551 ATCGTATAAA TTCATCATTT CAACCTCTTT ACGCGCTTCG TTAATACGAC
2601 TTTGTATTTT CTCATCAGAT TCTGTTCTCT TACCTACTAA TCGCTCTCTC
2651 AAGTGTTCTA AACTTGGAGG TGCTAAGAAA ATAAATAGCG CATCTGGAAA
2701 TTTCTTTCTA ACTTGCTTTG CACCTTCTAC TTCAATTTCT AAAAATACAT
2751 CATGACCTTC GTCCATTGTA TCTTTAACAT ATTGAACTGG TGTACCATAA
2801 TAGTTGCCCTA CATATTTCAGC ATATTCTATA AATTGGTCAT CTTTGATTAA

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2851 AGCTTCAAAC GCATCCCTAG TTTTAAAAA GTAATCTACG CCATTCAACW
2901 TCACCTTCAC GCATTTGACG TGTTGTCATT GGAATAGRAG AGCTTRANNG
2951 ATGTATNGNG ATCGACCTGC AGTCAT

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Mutant: NT124**phenotype:** temperature sensitivity

10 **Sequence map:** Mutant NT124 is complemented by plasmid pMP677, which carries a 3.0 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 60 with open boxes to depict the current status of the contig project; no apparent restriction sites for EcoR

15 I, Hind III, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal no significant similarities to known genes at this time.

20 **DNA sequence data:** The following DNA sequence data represents the sequence generated from clone pMP677, starting with standard M13 forward and M13 reverse sequencing primers; the sequence contig will be completed later via primer walking strategies. The sequence below

25 can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP677

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SEQ ID NO. 66

pMP677.forward Length: 540 nt

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1  TACCCGGGGA CCTTGAAAAA TACCTGGTGT ATCATAACATA AATGANGTGT
35  51  CATCTANAGG AATATCTATC ATATCTNAAG TTGTTCCAGG GANTCTTGAA
    101  GTTGTTACTA CATCTTTTTC ACCAACACTA GCTTCAATCA GTTTATTAAT
    151  CAATGTAGAT TTCCCAACAT TCGTTGTCCC TACAATATAC ACATCTTCAT
    201  TTTCTCGAAT ATTCGCAATT GATGATAATA AGTCNTNTNT GCCCCAGCCT
    251  TTTTCAGCTG AAATTAATAC GACATCGTCA GCTTCCAAAC CATATTTTCT
40  301  TGCTGTTTCG TTTAACCATT CTTTAACTCG ACGTTTATTA ATTTGTTTCG
    351  GCAATAAATC CAATTTATTT GCTGCTAAAA TGATTTTTTT GTTCCGACA
    401  ATACGTTTAA CTGCATTAAT AAATGATCCT TCAAAGTCAA ATACATCCAC
    451  GACATTGACG ACAATACCCT TTTTATCCGC AAGTCCTGAT AATAATTTTA
    501  AAAAGTCTTC ACTTTCTAAT CCTACATCTT GAACTTCGTT
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SEQ ID NO. 67

pMP677.reverse Length: 519 nt

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5      1  GACGCGTAAT TGCTTCATTG AAAAAATATA TTTGTNGAAA GTGGTGCATG
      51  ACAAATGTAC TGCTCTTTTT GTAGTGTATC AGTATTGTGA TGTTTTAATG
     101  AGAATATTAT ATGAATCATT ATGAAATTTA ATAAAAATAA AAGAAATGAT
     151  TATCATTTTT TCTTATATAC TGTAAACGG TTTGGAATTT TTAGGTATAC
     201  ACTGTATTGG TTGATATAAC TCAACTAATA ATTGCGAACA GAGTATTTCA
     251  AATTGAAAAG TATTATGAGC GTGATACATA ATCAAAATTG TAGGCTCAAG
    10  301  AACCCTACA TAATAACCA TAAGCGGTTT TTTATCATT ATGTCTCGCT
     351  CTCAAATGTA AATTAATAAT TGTTTTGGGG GAGTTTGAAG TTAAATATTT
     401  AACAGGATTT ATTTAATAT TATTGTTAGA AGGAATTTTT ACAAAATTCAG
     451  CGAGTGCAAT CGAATATTCA GACTTACATC ATAAAAGTAA GTTTGATTCA
    15  501  AAGCGTCCTA AGTTAATGC

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Mutant: NT125

20 Phenotype: temperature sensitivity

Sequence map: Mutant NT125 is complemented by plasmid pMP407, which carries a 3.3 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 61. Database searches at the nucleic acid and

25 (putative) polypeptide levels against currently available databases reveal strong peptide level similarities to *rnpA* (Genbank Accession No. X62539), encoding the protein component of RNaseP (EC 3.1.26.5), and *thdF* (Genbank Accession No. X62539), a hypothetical ORF with similarities

30 to the thiophene/furan oxidase from *E. coli*.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP407, starting with standard M13 forward and M13

35 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

40

clone pMP407

SEQ ID NO. 68

pMP407 Length: 3308 nt

45

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1  ACCAATATAT GCATCTGAAC GACTTAATAT CTTTTCGCCT GTGTTTAACA

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51 CTTTACCTGC AGCGTTAATA CCTGCCATCA ATCCTTGTCC TGCTGCTTCT
 101 TCATAACCAG ATGTACCATT AATTTGACCT GCAGTATATA AGTTTTTAAT
 151 CATTTCGTT TCAAGTGTAG GCCATAACTG CGTTGGCACA ATCGCATCAT
 201 ATTCAATTGC GTAGCCGGCA CGCATCATAT CTGCTTTTTC AAGACCTGGT
 251 ATCGTCTCTA ACATTTGACG TTGCACATGT TCAGGAAGAC TTGTNGACAA
 301 TCCTTGACACA TATACTTCAT TTGTATTAAC GACCTTCAGG CTCTAAGAAA
 351 AAGTTGATGT CGCGGCTTAT CATTAAATCG AACAAATTTA TCTTCAATTG
 401 AAGGGCAATA ACGTGGCCCG GTTCCTTTAA TCATCCCTGA ATACATTGCA
 451 GATAGATGTA AATTATCATC GATAACTTTG TGTGTTTCAN CATTAGTATA
 501 CGTTAGCCAA CATGGCAATT GATCKAMYAT ATATTCTGTT GTTTCAAAGC
 551 TGAATGCACG ACCTACATCG TCACCTGGTT GTATTTCAGT CTTCGAATAR
 601 TCAATTGTTT TTGAATTGTA CACGGCGGWG GTGTACCTGT TTTAAACGA
 651 ACAATATCAA AACCAAGTTC TCTTARATGK GKSTGATAAT GTGATTGATG
 701 GTAATTGGTG GATTTGGTCC ACTTGAATAC TTCATATTAC CTAAAATGAT
 751 TTCACCACGT ATRAAATGTT GCCCGTWGTA ATAATTACTG CTTTAGATAA
 801 ATACTCTGTA CCAATATTTG TACGTACACC TTKAACTGTC ATTAWCTTCT
 851 ATAACAAGTT CGTCTACCAT ACCTTGCAAT AATATGCAAA TTTTCTTCAT
 901 CTTCAATCAM GCGTTTCATT TCTTGTTGAT AAAGTACTWT AKCTGCTTGC
 951 GCCKCTWAGT GCTCTTACAR CAGGTCCTTT AACTGTATTT AACATTCTCA
 1001 TTTGAATGTG TGTTTTATCG ATTGTTTTTG CCATTTGTCC ACCTAAAGCA
 1051 TCAATTTTAC GAACAACGAT ACCTTTAGCT GGTCCACCTA CAGATGGGTT
 1101 ACATGGCATA AATGCAATAT TATCTAAAT TATTGTTAGC ATTAATGTTT
 1151 TAGCACCACG TCTTGACAGT GCTAAACCTG CTTCTACACC TGCATGTCCC
 1201 GCACCTATAA CGATTACATC ATATTCTTGA ACCACAATAT AAACCTCCTT
 1251 ATTTGATATC TTACTAGCCK TCTTAAGACG GTATTCCGTC TATTTCAATT
 1301 ACTATTTACC TAAGCAGAAT TGACTGAATA ACTGATCGAT GAGTTCATCA
 1351 CTTGCAGTCT CACCAATAAT TTCTCCTAAT ATTTCCCAAG TTCTAGTTAA
 1401 ATCAATTTGT ACCATATCCA TAGGCACACC AGATTCTGCT GCATCAATCG
 1451 CMTCTWGTAT CGTTTGTCTT GCTTGTTTTA ATAATGAAAT ATGTCTTGAA
 1501 TTAGAAACAT AAGTCATATC TTGATTTTTG TACTTCTCCA CCAAAGAACA
 1551 AATCTCGAAT TTGTATTTCT AATTCATCAA TACCTCCTTG TTTTAACATT
 1601 GAAGTTTGAA TTAATGGCGT ATCACCTATC ATATCTTTAA CTTCATTAAT
 1651 ATCTATGTTT TGCTCTAAAT CCATTTTATT AACAAATTACG ATTACATCTT
 1701 CATTTTAAAC CACTTCATAT AATGTGTAAT CTTCTTGAGT CAATGCTTCG
 1751 TTATTGTTTA ATACAAATAA AATTAAGTCT GCTTGGCTAA GAGCCTTTCT
 1801 AGAGCGTTCA ACACCAATCT TCTCTACTAT ATCTTCTGTC TCACGTATAC
 1851 CAGCAGTATC AACTAATCTT AATGGCACGC CACGAACATT GACGTAMTCT
 1901 TCTAAGACAT CTCTAGTAGT ACCTGCTACY TCAGTTACAA TCGCTTTATT
 1951 ATCTTGATTT AAATTATTTA ACATCGATGA TTTACCTACG TTTGGTTTAC
 2001 CAACAATAAC TGTAGATAAA CCTTCACGCC ATAATTTTAC CTTGCGCACC
 2051 GGTATCTAAT AAACGATTAA TTTCTGTTT GATTTCTTTA GACTGCTCTA
 2101 AAAGAAATTC AGTAGTCGCA TCTTCAACAT CATCGTATTC AGGATAATCA
 2151 ATATTCACCT CCACCTGAGC GAGTATCTCT AATATAGATT GCAGTTGTTT
 2201 TTTGATTAAAG TCACCTAGAC GACCTTCAAT TTGATTTCATC GCAACTTAG
 2251 AAGCTCTATC TGTCTTCGAG CGAWWAAAGT CCATAACTGY TTCAGCTTGA
 2301 GATAAATCAA TACGACCATT TAAAAAGGCA MGTTTTGTAATTTCAACCTG
 2351 GCTCAGCCAT TCTAGCGCCA TATGTCATAG TAAGTTCCAG CACTCTATTA
 2401 ATCGTTAAAA TACCACCATG ACAATTAATT TCTATAATAT CTTGCGGTGT
 2451 AAATGTTTTT GGCGCTCTTA ACACAGACAC CATAACTTNT TCAACCATTCT
 2501 TTTAGACTCT GGATCAATAA TATGACCGTA ATTAATCGTA TGTGATGGAA
 2551 CATCATTTAA AAGATGTTTT CTTTATATA ATTTGTCAGC AATTTCAACG

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2601 GCTTGCGGTC CAGACAATCG AACAATTCCA ATTGCCCTT CACCCATTGG
2651 TGTGAAATA CTCGTAATTG TATCTAAATC CATATTGCTA CTCGCCTCCT
2701 TCAACGATGT GAATACATTT TAAAGTAAGT TATTATAACC CTAAGGTCAG
2751 TCTTAACGTT TGTCTGAGGT AAGACTTCGG GATGTGTTGA GTGGTTAATG
5 2801 TTTTCCTTCC CCTACCCTAT CCTTACTTAA TCTTTTTATT AAAAATTG
2851 GCAATTTTAA GTACGTGCTC AAGACTATTC TGTATTTGTA AAGTCGTCAT
2901 ATCTTTAGCT GGCTGTCTTG CTATTACAAT AATATCTTTG GCCAATATAT
2951 GCGACTTATG TACTTTGAAA TTTTCACGTA TTGCTCTTTT AATCTTGTTT
10 3001 CTTAACACTG CATTACCTAG TTTTGTAGAA ACACTAATAC CTAAGCGAAA
3051 ATGGTCTATT TCTTTATTAT TACAAGTGTA TACAACAAAT TGTCTGTTGG
3101 CTACAGAATG ACCTTTTTTA TATATTCTCT GAAAATCTGC ATTCTTTTTA
3151 ATTCGGTAAG CTTTTTCCAA TAACATCACT CGCTTATTTA TCGTTTTTAT
3201 TTGAAGCTAT ATTTAACTT CTATTGAGCT TATAACATAA ATTTCTATTT
3251 ATTCTTAATT TAAACGAAAA AAAAGATCGA CTCTAGAGGA TCCCCGGGTA
15 3301 CCGAGCTC

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Mutant: NT144

20 Phenotype: temperature sensitivity

Sequence map: Mutant NT144 is complemented by plasmid pMP414, which carries a 4.5 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 62. Database searches at the nucleic acid and

25 (putative) polypeptide levels against currently available databases reveal identity to the Hsp70 locus from *S. aureus* (Genbank Accession No. D30690), including an additional 600 bp of unpublished sequence upstream of the Genbank entry. Experiments are underway to determine which ORF in this

30 contig is the essential gene.

DNA sequence data: The following DNA sequence data represents the sequence generated from clone pMP414, starting with standard M13 forward and M13 reverse

35 sequencing primers; the sequence contig will be completed later via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

40

clone pMP414

SEQ ID NO. 69

pMP414.forward Length: 1004 nt

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      1 AGTTACGGCT TAATACTTGA ACCNAAAACC CAATTTTATA ATATGTATAG
    51 AAAAGGCTTG CTCAAACCTG CTAATGAGGA TTTAGGTGCT GACATGTATC
   101 AGTTGCTGAT GTCTAANATA GAACAATCTC CTTTCCATCA ATACGAAATA
   151 TCTAATTTTG CATTAGATGG CCATGANTCN NAACATAATA AGGTTTACTG
    201 GTTTAATGAG GAATATTATG GATTTGGAGC AGGTGCAAGT GGTTATGTAN
   251 ATGGTGTGCG TTATACGAAT ATCAATCCAG TGAATCATT TATCAAAGCT
   301 ATNAATAAAG AAAGTAAAGC AATTTTAGTA TCAAATAAAC CTTCTTTGAC
   351 TGAGAGAATG GAAGAAGAAA TGTTCCTTGG GTTGCCTTTA AATGAAAGTG
   401 TGAGTAGTAG TAGGTTCAAA AAGAAGTTTG ACCAATCTAT TGAAAGTGTC
  10 451 TTTGGTCAAA CAATAAATAA TTTAAAAGAG AAGGAATTAA TTGTAGAAAA
   501 AGAACGATGT GATTGCACTT ACAAATAGAG GGAAAGTCAT ANGTAATGAG
   551 GTTTTTGAAG CTTTCCTAAT CAATGATTAA GAAAAATTGA AATTTGAGT
   601 CTTTAACATT GACTTANTTT GACCAATTTG ATAAATTATA ATTAGCACTT
   651 GAGATAAGTG AGTGCTAATG AGGTGAAAAC ATGANTACAG ATAGGCAATT
  15 701 GAGTATATTA AACGCAATTG TTGAGGATTA TGTTGATTTT GGACAACCCG
   751 TTGGTTCTAA AACACTAATT GAGCGACATA ACTTGAATGT TAGTCCTGCT
   801 ACAATTAGAA ATGAGATGAA ACAGCTTGAA GATTTAAACT ATATCGAGAA
   851 GACACATAGT TCTTCAGGGC GTTCGCCATC ACAATTAGGT TTTAGGTATT
   901 ATGTCAATCG TTTACTTGAA CAAACATCTC ATCAAAAAAC AAATAAATTA
  20 951 AGACGATTAA ATCAATTGTT AGTTGAGAAC AATATGATGT TTCATCAGCA
    1001 TTGA

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SEQ ID NO. 70

pMP414.reverse Length: 1021 nt

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  25 1 CCTGCAGGTC GATCCTGACA ACATTCTAAT TGTATTGTTT AATTATTTTT
    51 TGTCGTCGTC TTTTACTTCT TTAAATTCAG CATCTTCTAC AGTACTATCA
   101 TTGTTTTGAC CAGCATTAGC ACCTTGCTGCT TGTGTTGCT GTTGAGCCGC
   151 TTGCTCATAT ACTTTTGCTG ATAATTCCTG AATCACTTTT TCAAGTTCTT
  30 201 CTTTTTTAGA TTAAATATCT TCTATATCTT GACCTTCTAA AGCAGTTTTA
   251 AGAGCGTCTT TTTTCTCTTC AGCAGATTTT TTATCTTCTT CACCGATATT
   301 TTCGCCTAAA TCAGTTAAAG TTTTTTCAAC TTGGAATACT AGACTGTCAG
   351 CTTGCTTTCT TAAGTCTACT TCTTCACGAC GTTTTTTATC TGCTTCAGCG
   401 TTAACCTCAG CATCTTTTAC CATAACGGTCR ATTTCTTCGT CTGATAATGA
  35 451 AGAACTTGAT TGAATTGTAA TTCTTTGTTT TTTATTTGTA CCTAAGTCTT
   501 TTGGCAGTTA CATTTACAAT ACCGTTTTTA TCGATATCAA ACGTTACTTC
   551 AATTTGGAGG TTTACCACCG TTTCAARMWGG TGGAATATCA GTCAATTGGA
   601 ATCTACCAAG TGTTTTATTA TCCGCAGCCA TTGGACGTTT ACCTTGTAAT
   651 ACGTGACAT CTAAGTATGG TTGATTATCT ACTGCTGTTG AATAGATTTG
  40 701 AGATTTAGAT GTAGGAATCG TAGTGTTACG TTCAATTAAC GTATTCATAC
   751 GTCCACCTAA AATTTCAATA CCTAAAGATA GTGGTGTTAC GTCTAATAAT
   801 ACTACGTCTT TAACGTCACC TGTGATAACG CCACCTTGGA TTGCAGCTCC
   851 CATTGCCACT ACTTCGTCCG GGTTTACTCC TTTGTTAGGC TCTTTACCGA
   901 TTTCTTTTTT GACAGCTTCT TGTACTGCTG GAATACGAAT TGATCCACCA
  45 951 ACTAAGATAA CTTTCATCGAT ATCTGANTTT GTTAAGCCAG CGTCTTTTCAT
    1001 TGCTTGGCGT GTAGGTCCAT C

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50 Mutant: NT152

Phenotype: temperature sensitivity

Sequence map: Mutant NT152 is complemented by plasmid pMP418, which carries a 3.0 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 63. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal limited peptide-level similarity to *yacF*, a hypothetical ORF, from *B. subtilis* (Genbank Accession No. D26185).

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP418, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP418

SEQ ID NO. 71

pMP418 Length: 3010 nt

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25      1 ATGCCTGCAG GTCGATCACG ATGNAAGTCA TTCAATAAGA ATGATTATGA
      51 AAATAGAAAC AGCAGTAAGA TATTTTCTAA TTGAAAATCA TCTCACTGCT
     101 GTTTTTTTAA GGTTTTATACC TCATCCTCTA AATTATTTAA AAATAATTAA
     151 TGGTATTTGA GCACGTTTAG CGACTTTATG ACTGACATTA CCAATTTCCA
     201 TTTCTTGCCA GATATTCAAA CCACGTGTAC TCAAAATGAT AGCTTGGTAT
30     251 GTACCTCCAA TAGTAATTC AATAACTTTG TCTGTTGAAC ACTAAGAGCA
     301 ATTTTAATTT CATAATGTGT TGTAACATT TTTTGTGATT GGAGTTTTTT
     351 TCTGAGTTAA ACGATATCCT GATGTATTTT TAATTTTGCA CCATTTCCAA
     401 AAGGATAAGT GACATAAGTA AAAAGGCATC ATCGGGAGTT ATCCTATCAG
     451 GAAAACCAAG ATAATACCTA AGTAGAAAAG TGTTCATCC GTGTTAAATT
35     501 GGGAAATATC ATCCATAAAC TTTATTACTC ATACTATAAT TCAATTTTAA
     551 CGTCTTCGTC CATTTGGGCT TCAAATTCAT CGAGTARTGC TCGTGCTTCT
     601 GCAATTGATT GTGTGTTTCA CAATTGATGT CGAAGTTCGC TAGCGCCTCT
     651 TATGCCACGC ACATAGATTT TAAAGAATCT ACGCAAGCTC TTGAATTGTC
     701 GTATTTTCATC TTTTTCATAT TTGTTAAACA ATGATAAATG CAATCTCAAT
40     751 AGATCTAATA GTTCCTTGCT TGTGTGTTTC CGTGGTCTT TTTCAAAGC
     801 GAATGGATTG TGGAAAATGC CTCTACCAAT CATGACGCCA TCAATGCCAT
     851 ATTTTTCTGC CAGTTCAAGT CCTGTTTTTC TATCGGGAAT ATCACCCTTA
     901 ATTGTTAACA ATGTATTTGG TGCAATTCG TCACGTAAAT TTTTAATAGC
     951 TTCGATTAAT TCCCAATGTG CATCTACTTT ACTCATTTCT TTACGTTGTA
45    1001 CGAAGATGAA TAGATAAATT GGCAATGTCT TGTTCGAAGA CAKTGCTTCA
    1051 ACCAATCTTT CCATTCATCG ATTCATAKT AGCCAAGGCG TGTTTTTTAA
  
```

5 1101 ACTTTACCGG AASCCACCT GCTTTAGTCG CTTGAATAAT TTCGGCAGCA
 1151 ACGTCAGGTC TTAAGATTAA GCCGGANCCC TTACCCTTTT TAGCAACATT
 1201 TGCTACAGGA CATCCCATAT TTAAGTCTAT GCCTTTAAAG CCCATTTTAG
 1251 CTAATTGAAT ACTCGTTTCA CGGAACTGTT CTGGCTTATC TCCCCATATA
 1301 TGAGCGACCA TCGGCTGTTT ATCTTCACTA AAAGTTAAGC GTCCGCGCAC
 1351 ACTATGTATG CCTTCAGGGT GGCAAAAGCT TTCAGTATTT GTAAATTCAG
 1401 TGAAAAACAC ATCCRGCTA GNTGCTTCAN TTACAACGTG TCGAAAGACG
 1451 ATATCTGTAA CGTCTTCCAT TGGCGCCAAA ATAAAAAATG GACGTGGTAA
 1501 TTCACTCCAA AAATTTTCTT TCATAATATA TTTATACCTT CTTTATAATT
 10 1551 AGTATCTCGA TTTTTTATGC ATGATGATAT TACCACAAAA GCNTAACTTA
 1601 TACAAAAGGA ATTTCAATAG ATGCAACCAT TKGAAAAGGG AAGTCTAAGA
 1651 GTAGTCTAAA ATAAATGTTG TGGTAAAGTT ATCAATACAA AGATCAAGGA
 1701 TTATAGTATT AAATTGTTCA TTATTAATGA TACTACTT ATGAATATGA
 1751 TTCAGAATTT TCTTTGGCTA CTNCTTACAG TAAAGCGACC TTTTAGTTAT
 15 1801 CTTATAACAA AGACAAATTT CTAAAGGTGA TATTATGGAA GGTTTAAAGC
 1851 ATTCTTTAAA AAGTTTAGGT TGGTGGGATT NATTTTTTGC GATACCTATT
 1901 TTTCTGCTAT TCGCATACCT TCCAACTNT AATTTTATA NCATATTTCT
 1951 TAACATTGTT ATCATTATTT TCTTTCCNT AGGTTTGATT TTAACACGC
 2001 ATATAATTAT AGATAAAAYT AAGAGCAACA CGAAATGAAT CATTAAACG
 20 2051 GAATGTGATT AAAACATAAA ACTGAAGGAG CGATTACAAT GGCGACTAAG
 2101 AAAGATGTAC ATGATTTTATT TTTAAATCAT GTGAATTCAA ACGCGGTAA
 2151 GACAAGAAAG ATGATGGGAG AATATATTAT TTATTATGAT GGCGTGGTTA
 2201 TAGGTGGTTT GTATGATAAT AGATTATTGG TCAAGGCGAC TAAAAGTGCC
 2251 CAGCAGAAAT TGCAAGATAA TACATTAGTT TCGCCATATC CAGGTTTCTA
 25 2301 AAGAAATGAT ATTAATTTTA GACTTTACCG AAGCAACAAA TCTCACTGAT
 2351 TTATTTAAGA CCATAAAAAA TGATTTGAAA AAGTGAAGTA GTGAAGTGTG
 2401 GGTGCAGAGA GAACTAAGCC CATCGWTAAT TGGTCGCTTG TTAAAGAAGA
 2451 GTGACGGTCA CTCTTCTTTA TGTGCATATT TTATTTTGTC TGTTTBGTTA
 2501 ACAAGCAGCA GTGTAACAAA TATGAGTAAG GATAAAATGA GTATAATATA
 30 2551 GAAACCGAAT TTATCATTA TTTTATTAAT CCATCTTCCT AAAAATGGAG
 2601 CAATTAACT TTGCAGTAAC AATGAAATTG ACGTCCATAT CGTAAATGAG
 2651 CGACCGACAT ATTTATCTGA AACAGTGTTT ATTATAGCWG TATTATATA
 2701 AATTCTGATT GATGAAATTG AGTAGCCTAG TATAAAKGAT CCTATGAATA
 2751 AGTAAAATGC TGAGTTTATC CAAATAAATA GTGCKGAATT TATGACTRRC
 35 2801 TATGAAATAT AACAAAAATA TCACATACTT TAGKTGAGAT TTTCTTSGAA
 2851 AGAATAGCTG AAATTAAACC TGCACATAAT CCTCCAATGC CATATAACAT
 2901 ATCTGAAMAA CCAAATGTA CAGACCGAAA GTTTTAAAC ATTATAACA
 2951 TATCCTGGTA ATGATATGTT AAAGATCGAC TCTAGAGGAT CCCC GGNTAC
 3001 CGAGCTCGAA

40

Mutant: NT156

45 **phenotype:** temperature sensitivity

Sequence map: Mutant NT156 is complemented by plasmids pMP672 and pMP679, which carry 4.5 kb inserts of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 64. Database searches at the nucleic acid

and (putative) polypeptide levels against currently available databases reveal identity to the *grlBA* locus, a known essential gene encoding DNA topoisomerase (EC 5.99.1.3), from *S. aureus* (Genbank Accession No. L25288; published in Ferrero, L. et al. *Mol. Microbiol.* 13 (1994) 641-653).

DNA sequence data: The following DNA sequence data represents the sequence generated from clone pMP679, starting with standard M13 forward and M13 reverse sequencing primers; the sequence contig will be completed later via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clones pMP679 and pMP672

SEQ ID NO. 72

20 pMP679.forward Length: 548 nt

```

1  ATCGGTACCC GGGGACCAAT ANACAGAAAG TATATTAAGT TTNGTAAATA
51  ATGTACGTAC TNAAGATGGT GGTACACATG AAGTTGGTTT TAAACAGCA
101  ATGACACGTG TATTTAATGA TTATGCACGT CGTATTAATG AACTTAAAC
25  151  AAAAGATAAA AACTTAGATG GTAATGATAT TCGTGAAGGT TTAACAGCTG
201  TTGTGTCTGT TCGTATTCCA GAAGAATTAT TGCAATTGTA ANGACAAACG
251  AAATCTAAAT TGGGTACTTC TGAAGCTAGA AGTGCTGTTG ATTCAGTTGT
301  TGCAGACAAA TTGCCATTCT ATTTAGAAGA AAAAGGACAA TTGTCTAAAT
351  CACTTGTGGA AAAAAGCGAT TAAAGCACAA CAAGCAAGGG AAGCTGCACG
30  401  TAAAGCTCGT GAAGATGCTC GTTCAGGTAA GAAAAACAAG CGTAAAGACA
451  CTTTGCTATC TGGTAAATTA ACACCTGCAC AAAGTTAAAA AACTTGGA
501  AAAATGAATT GTATTTAGTC GAAGGTGATT CTGCGGAAG TTCAGCAA

```

SEQ ID NO. 73

35 pMP679.reverse Length: 541 nt

```

1  ACTGCAGGTC GAGTCCAGAG GWCTAAATTA AATAGCAATA TTAATAAAC
51  CATACCAATG TAAATGATAG CCATAATCGG TACAATTAAC GAAGATGACG
101  TAGCAATACT ACGTACACCA CCAAATATAA TAATAGCTGT TACGATTGCT
40  151  AAAATAATAC CTGTGATTAC TGGACTAATA TTATATTGCG TATTTAACGA
201  CTCCGCAATT GTATTAGATT GCACTGTGTT AAATACAAAT GCAAATGTAA
251  TTGTAATTAA AATCGCAAAT ACGATACCTA GCCATTTTGT ATTTAAACCT
301  TTAGTAATAT AGTAAGCTGG ACCACCACGG GAATCCACCA TCTTTATCAT
351  GTACTTTATA AACCTGAGCC AAAGTCGCTT CTATAAATGC ACTCGCTGCA
45  401  CCTATAAATG CAATAACCCA CATCCAAAAT ACTGCACCTG GACCGCCTAA
451  AACAAATCGCA GTCGCAACAC CAGCAATATT ACCAGTACCA ACTCTCGAAC
501  CAGCACTAAT CGCAAATGCT TGAATGGCG AAATACCCTT C

```

SEQ ID NO. 74

pMP672.forward Length: 558 nt

```

5      1  AGGGTCTNNC  ACGGTACCCG  GGGNCCAATT  WGATGAGGAG  GAAATCTAGT
      51  GAGTGAAATA  ATKCAAGATT  TATCACTTGA  AGATGTTTTA  GGTGATCGCT
     101  TTGGAAGATA  TAGTAAATAT  ATTATTCAAG  AGCGTGCATT  GCCAGATGTT
     151  CGTGATGGTT  TAAAACCAGT  ACAACGTCGT  ATTTTATATG  CAATGTATTC
     201  AAGTGGTAAT  ACACACGATA  AAAATTTCCG  TAAAAGTGCG  AAAACAGTCG
10     251  GTGATGTTAT  TGGTCAATAT  CATCCACATG  GGAGACTCCT  CAGTGTACGA
     301  AGCAATGGTC  CGTTTAAGTC  AAGACTGGAA  GTTACGACAT  GTCTTAATAG
     351  AAATGCATGG  TAATAATGGT  AGTATCGATA  ATGATCCGCC  AGCGGCAATG
     401  CGTTACACTG  AAGCTAAGTT  AAGCTTACTA  GCTGAAGAGT  TATTAGCTGA
     451  TATTAATAAA  GAGACAGTTT  CTTTCATTCC  AAACATATGAT  GATACGACAC
15     501  TCCGAACCAA  TGGTATTGCC  ATCAAGAATT  TCCTAACTTA  CTAAKTGAAT
     551  GTTTCTAC

```

20 **Mutant:** NT160

Phenotype: temperature sensitivity

Sequence map: Mutant NT160 is complemented by plasmid pMP423, which carries a 2.2 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 65. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal identity to the *Dlt* locus of *S. aureus* (Genbank Accession No. D86240; unpublished). The pMP423 clone completely contains the genes *dltC*, encoding a putative D-Alanine carrier protein, and *dltD*, encoding a putative "extramembranal protein". Further subcloning and recombination experiments already in progress will demonstrate whether one or both of the ORFs encode essential genes.

35

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP423, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

45 clone pMP423

SEQ ID NO. 75

pMP423 Length: 2234 nt

```
5      1 AGTCGATCTT TATTCTACAT GTCTCGTAAA AAATTATTGA AGAGTCAATT
      51 TGCAATGTCT AACGTGGCAT TCTTAATCAA CTTCTTCATA ATGGGAATTT
     101 GGCATGGTAT CGAAGTGTAT TACATTGTTT ATGGTTTATA CCATGCAGCA
     151 TTGTTTATAG GTTATGGCTA TTATGAACGT TGGCGTAAGA AACATCCGCC
     201 ACGTTGGCAA AATGGTTTCA CAACAGCACT TAGCATTGTG ATTACATTCC
10     251 ACTTTGTAAC ATTTGGCTTT TTAATCTTCT CAGGTAAACT TATATAATAA
     301 AGGAGAATTT AATTATGGAA TTTAGAGAAC AAGTATTAAA TTTATTAGCA
     351 GAAGTAGCAG AAAAATGATA TTGTAAAAGA AAATCCAGAC GTAGAAATTT
     401 TTGAAGAAGG TATTATTGAT TCTTTCCAAA CAGTTGGATT ATTATTAGAG
     451 ATTCAAAATA AACTTGATAT CGAAGTATCT ATTATGGACT TTGATAGAAG
15     501 ATGAGTGGGC MACACCAAAT AAAATCGTTG AAGCATTAGA AGAGTTACGA
     551 TGAAATTAAG ACCTTTTTTA CCCATTTTAA TTAGTGGAGC GGTATTCAAT
     601 GTCTTTCTAT TATTACCTGC TAGTTGGTTT ACAGGATTAG TAAATGAAAA
     651 GACTGTAGAA GATAATAGAA CTTCAATGAC AGATCAAGTA CTAAGGCA
     701 CACTCAWTCA AGATAAGTTA TACGAATCAA ACAAGTATTA TCCTATATAC
20     751 GGCTCTAGTG AATTAGGTAA AGATGACCCA TTTAATCCTG CAATTGCATT
     801 AAATAAGCAT AACGCCAACA AAAAAGCATT CTTATTAGGT GCTGGTGGTT
     851 CTACAGACTT AATTAACGCA GTTGAAGTTG CATCACAGTT ATGATAAATT
     901 AAAAGGTTAA GAAATTAACA TTTATTATTT CACCACAATG GTTTACAAAC
     951 CCATGGTTTA ACGAATCCAA AACTTTGATG CTCSTATGTC TCAAACCTMA
25    1001 ATTAATCAAA TGTTCCTASC AGAAAAACAT GTCTACTGAA TTAAACGTC
     1051 GTTATGCACA ACGTTTATTA CAGTTTCCAC ATGTACACAA TAAAGAATAC
     1101 TTGAAATCTT ATGCTAAAAA CCTAAAGAA ACTAAAGRTA GTTATATTTT
     1151 TGGKTTTWAA RAGAGATCAA TTGATTAAAA TAGAAGCGAT TAAATCATTG
     1201 TTTGCAATGG ATAAATCTCC ATTAGAACAT GTTAAACCTT GCTACAAAAC
30    1251 CAGACGCTTC TTGGGATGAG ATGAAACAAA AAGCAGTTGA AATTGGTAAA
     1301 GCTGATACTA CATCGAATAA ATTTGGTATT AGAGATCAAT ACTGGAAATT
     1351 AATTCCAAGA AAGTAAGCCG TTAAAGTTAG ACGTTGACTA CGAATTCMAT
     1401 GTTWATTCTC CCAGAATTCC MAGATTTAGA ATTACTTGTW AAAAMMATGC
     1451 KTGCTGCTGG TGCAGATGTT CAATATGTAA GTATTCCATC AAACGGTGTA
35    1501 TGGTATGACC ACATTGGTAT CGATAAAGAA CGTCGTCAAG CAGTTTATAA
     1551 AAAAATCCAT TCTACTGTTG TAGATAATGG TGGTAAAATT TACGATATGA
     1601 CTGATAAAGA TTATGAAAAA TATGTTATCA GTGATGCCGT ACACATCGGT
     1651 TGGAAAGGTT GGGTTTATAT GGATGAGCAA ATTGCGAAAC ATATGAAAGG
     1701 TGAACCACAA CCTGAAGTAG ATAAACCTAA AAATTAAAT ACAAATAGCA
40    1751 CATAACTCAA CGATTTTGAT TGAGCGTATG TGCTATTTT ATATTTTAAA
     1801 TTTCATAGAA TAGAATAGTA ATATGTGCTT GGATATGTGG CAATAATAAA
     1851 ATAATTAATC AGATAAATAG TATAAAATAA CTTTCCCATC AGTCCAATTT
     1901 GACAGCGAAA AAAGACAGGT AATAACTGAT TATAAATAAT TCAGTATTCC
     1951 TGTCTTTGTT GTTATTCATA ATATGTTCTG TTAAGTTAAT ATCTTTATAT
45    2001 TAGAATACTT GTTCTACTTC TATTACACCA GGCATTCTT CGTGTAATGC
     2051 ACGCTCAATA CCAGCTTTAA GAGTGATTGT AGAACTTGGG CATGTACCAC
     2101 ATGCACCATG TAATTGTAAT TTAACAATAC CGTCTTCAC GTCAATCAAT
     2151 GAGCAGTCGC CACCATCAG TAATAAAAAT GGACGAAGAC GTTCAATAAC
     2201 TTCTGCTACT TGATCGACCT GCAGGCATGC AAGC
```

Mutant: NT166

Phenotype: temperature sensitivity

Sequence map: Mutant NT166 is complemented by plasmid
 5 pMP425, which carries a 3.3 kb insert of wild-type *S.*
aureus genomic DNA. A partial restriction map is depicted
 in Fig. 66. Database searches at the nucleic acid and
 (putative) polypeptide levels against currently available
 databases reveal strong peptide-level similarities to *nrdE*,
 10 encoding ribonucleotide diphosphate reductase II (EC
 1.17.4.1), from *B. subtilis* (Genbank Accession No. Z68500),
 and *ymaA*, a hypothetical ORF, from *B. subtilis* (same
 Genbank entry).

15 **DNA sequence data:** The following DNA sequence data
 represents the sequence generated by primer walking through
 clone pMP425, starting with standard M13 forward and M13
 reverse sequencing primers and completing the sequence
 contig via primer walking strategies. The sequence below
 20 can be used to design PCR primers for the purpose of
 amplification from genomic DNA with subsequent DNA
 sequencing.

clone pMP425

25

SEQ ID NO. 76

pMP425 Length: 3305 nt

	1	GAGCTCGGTA	CCCGGGGATC	CTCTAGAGTC	GATCCAATGA	AAATAATATA
30	51	TTTTTCATTT	ACTGGAAATG	TCCGTCGTTT	TATTAAGAGA	ACAGAACTTG
	101	AAAATACGCT	TGAGATTACA	GCAGAAAATT	GTATGGAACC	AGTTCATGAA
	151	CCGTTTATTA	TCGTTACTGG	CACTATTGGA	TTTGGAGAAG	TACCAGAACC
	201	CGTTCAATCT	TTTTTAGAAG	TTAATCATCA	ATACATCAGA	GGTGTGGCAG
	251	CTAGCGGTAA	TCGAAATTGG	GGACTAAATT	TCGCAAAGC	GGGTCGCACG
35	301	ATATCAGAAG	AGTATAATGT	CCCTTTATTA	ATGAAGTTTG	AGTTACATGG
	351	GAAAAACAA	AGACGTTATT	GAATTTAAGA	ACAAGGTGGG	TAATTTTAAT
	401	GAAACCATG	GAAGAGAAAA	AGTACAATCA	TATTGAATTA	AATAATGAGG
	451	TCACTAAACG	AAGAGAAGAT	GGATTCTTTA	GTTTAGAAAA	AGACCAAGAA
	501	GCTTTAGTAG	CTTATTTAGA	AGAAGTAAAA	GACAAAACAA	TCTTCTTCGA
40	551	CACTGAAATC	GAGCGTWTAC	GTTMTTTAGT	AGACMACGAT	TTTTATTTCA
	601	ATGTGTTTGA	TATWTATAGT	GAAGCGGATC	TAATTGAAAT	CACTGATTAT
	651	GCAAAATCAA	TCCCGTTTAA	TTTGTCAAGT	TATATGTCAG	CTAGTAAATT
	701	TTTCAAAGAT	TACGCTTTGA	AAACAAATGA	TAAAAGTCAA	TACTTAGAAG
	751	ACTATAATCA	ACACGTTGCC	ATTGTTGCTT	TATACCTAGC	AAATGGTAAT
45	801	AAAGCACAAAG	CTAAACAATT	TATTTCTGCT	ATGGTTGAAC	AAAGATATCA

	851	ACCAGCGACA	CCAACATTTT	TAAACGCAGG	CCGTGCGCGT	TCGTGGTGGA
	901	GCTAGTG TTC	ATTGTTTCCT	TATTAGAAGT	TGGATGGACA	GCTTAAATTC
	951	AATTTAACTT	TATTGGATTC	AACTGCAAAA	CAATTAAGTW	AAATTGGGGG
	1001	CGGSGTTTGC	MATTAACCTA	TCTAAATTGC	GTGCACGTGG	TGAAGCAATT
5	1051	AAAGGAATTA	AAGGCGTAGC	GAAAGGCGTT	TTACCTATTG	CTAAGTCACT
	1101	TGAAGGTGGC	TTTAGCTATG	CAGATCAACT	TGGTCAACGC	CCTGGTGCTG
	1151	GTGCTGTGTA	CTTAAATATC	TTCCATTATG	ATGTAGAAGA	ATTTT TAGAT
	1201	ACTAAAAAAG	TAAATGCGGA	TGAAGATTTA	CGTTTATCTA	CAATATCAAC
	1251	TGGTTTAATT	GTTCCATCTA	AATTCTTCGA	TTTAGCTAAA	GAAGGTAAGG
10	1301	ACTTTTATAT	GTTTGCACCT	CATACAGTTA	AAGAAGAATA	TGGTGTGACA
	1351	TTAGACGATA	TCGATTTAGA	AAAAATATTAT	GATGACATGG	TTGCAAAACC
	1401	AAATGTTGAG	AAAAAGAAAA	AGAATGCGCG	TGAAATGTTG	AATTTAATTG
	1451	CGCMAACACA	ATTACAATCA	GGTTATCCAT	ATTTAATGTT	TAAAGATAAT
	1501	GCTAACAGAG	TGCATCCGAA	TTCAAACATT	GGACAAATTA	AAATGAGTAA
15	1551	CTTATGTACG	GAAATTTTCC	AACTACAAGA	AACTTCAATT	ATTAATGACT
	1601	ATGGTATTGA	AGACGAAATT	AAACGTGATA	TTTCTTGTA	CTTGGGCTCA
	1651	TTAAATATTG	TTAATGTAAT	GGAAAGCGGA	AAATTCAGAG	ATTCAGTTCA
	1701	CTCTGGTATG	GACGCATTAA	CTGTTGTGAG	TGATGTAGCA	AATATTCAAA
	1751	ATGCACCAGG	AGTTAGAAAA	GCTAACAGTG	AATTACATTC	AGTTGKTCTT
20	1801	GGGTGTGATG	AATTWACACG	GTTACCTAGC	AAAAAATAAA	ATTGGTTATG
	1851	AGTCAGAAGA	AGCAAAAGAT	TTTGCAAATA	TCTTCTTTAT	GATGATGAAT
	1901	TTCTACTCAA	TCGAACGTTT	AATGGAAATC	GCTAAAGAGC	GTGGTATCAA
	1951	ATATCAAGAC	TTTGAAAAGT	CTGATTATGC	TAATGGCAAA	TATTTTCGAGT
	2001	TCTATACAAC	TCAAGAATTT	GAACCTCAAT	TCGAAAAAGT	ACGTGAATTA
25	2051	TTCGATGGTA	TGGCTATTCC	TACTTCTGAG	GATTGGAAGA	AACTACAACA
	2101	AGATGTTGAA	CAATATGGTT	TATATCATGC	ATATAGATTA	GCAATTGCTC
	2151	CAACACAAAG	TATTTCTTAT	GTTCAAAATG	CAACAAGTTC	TGTAATGCCA
	2201	ATCGTTGACC	AAATTGAACG	TCGTA CTTAT	GGTAAATGCG	GAAACATTTT
	2251	ACCCTATGCC	ATTCTTATCA	CCACAAACAA	TGTGGTACTA	CAAATCAGCA
30	2301	TTCAATACTG	ATCAGATGAA	ATTAATCGAT	TTAATTGCGA	CAATTCAAAC
	2351	GCATATTGAC	CAAGGTATCT	CAACGATCCT	TTATGTTAAT	TCTGAAATTT
	2401	CTACACGTGA	GTTAGCAAGA	TTATATGTAT	ATGCGCACTA	TAAAGGATTA
	2451	AAATCACTTT	ACTATACTAG	AAATAAATTA	TTAAGTGTAG	AAGAATGTAC
	2501	AAGTTGTTCT	ATCTAACAA	TAAATGTTGA	AAATGACAAA	CAGCTAATCA
35	2551	TCTGGTCTGA	ATTAGCAGAT	GATTAGACTG	CTATGTCTGT	ATTTGTCAAT
	2601	TATTGAGTAA	CATTACAGGA	GGAAATTATA	TTCATGATAG	CTGTTAATTG
	2651	GAACACACAA	GAAGATATGA	CGAATATGTT	TTGGAGACAA	AATATATCTC
	2701	AAATGTGGGT	TGAAACAGAA	TTTAAAGTAT	CAAAAGACAT	TGCAAGTTGG
	2751	AAGACTTTAT	CTGAAGCTGA	ACAAGACACA	TTTAAAAAAG	CATTAGCTGG
40	2801	TTTAACAGGC	TTAGATACAC	ATCAAGCAGA	TGATGGCATG	CCTTTAGTTA
	2851	TGCTACATAC	GACTGACTTA	AGGAAAAAAG	CAGTTTATTC	ATTTATGGCG
	2901	ATGATGGAGC	AAATACACGC	GAAAAGCTAT	TCACATATTT	TCACAACACT
	2951	ATTACCATCT	AGTGAACAA	ACTACCTATT	AGATGAATGG	GTTT TAGAGG
	3001	AACCCCATTT	AAAATATAAA	TCTGATAAAA	TTGTTGCTAA	TTATCACAAA
45	3051	CTTTGGGGTA	AAGAAGCTTC	GATATACGAC	CAATATATGG	CCAGAGTTAC
	3101	GAGTGTATTT	TTAGAAACAT	TCTTATTCTT	CTCAGGTTTC	TATTATCCAC
	3151	TATATCTTGC	TGGTCAAGGG	AAAATGACGA	CATCAGGTGA	AATCATTCGT
	3201	AAAATTCTTT	TAGATGAATC	TATTCATGGT	GTATTTACCG	GTTTAGATGC
	3251	ACAGCATTTA	CGAAATGAAC	TATCTGAAAG	TGAGAAACAA	AAAGCAGATC
50	3301	GACCT				

Mutant: NT 199

Phenotype: temperature sensitivity

5 **Sequence map:** Mutant NT199 is complemented by plasmid pMP642, which carries a 3.6 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 67. Database searches at the nucleic acid and (putative) polypeptide levels against currently available
10 databases reveal strong peptide-level similarities to *yybQ*, an uncharacterized ORFs identified in *B. subtilis* from genomic sequencing efforts.

DNA sequence data: The following DNA sequence data
15 represents the sequence generated by primer walking through clone pMP642, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of
20 amplification from genomic DNA with subsequent DNA sequencing.

clone pMP642

25 **SEQ ID NO.** 77

pMP642 Length: 1945 nt

```

      1  TTGATAGTTT ATTGGAGAGA AAGAAGTATT AATCAAGTCG AAATCGTTGG
      51  TGTATGTACC GATATTTGCG TGTTACATAC AGCAATTTCT GCATACAAC
30    101  TAGGTTATAA AATTTTCAGTA CCTGCTGAGG GAGTGGCTTC ATTTAATCAA
      151  AAAGGGCATG AATGGGCACT TGCACATTTT AAAAACTCAT TAGGTGCAGA
      201  GGTAGAACAA CACGTTTAAA TCGTGCTAAA ATAATTATAA AGAATACAAT
      251  TTACAAGGGA GATATTTGAC AATGGCTAAA ACATATATTT TCGGACATAA
      301  GAATCCAGAC ACTGATGCAA TTTCATCTGC GATTATTATG GCAGAATTTG
35    351  AACAACTTCG AGGTAATTCA GGAGCCAAAG CATAACGTTT AGGTGATGTG
      401  AGTGCARAAA CTCAATTTCG GTTAGATACA TTTAATGTAC CTGCTCCGGA
      451  ATTATTAACA GATGATTTAG ATGGTCAAGA TGTTATCTTA GTTGATCATA
      501  ACGAATTCCA ACAAAGTTCT GATACGATTG CCTCTGCTAC AATTAAGCAT
      551  GTAATTGATC ATCAGAGAAT TGCAAATTTT GAAACTGCTG GTCCTTTATG
40    601  TTATCGTGCT GAACCAGTTG GTTGACAGC TACAATTTTA TACAAAATGT
      651  TTAGAGAACG TGGCTTTGAA ATTAAACCTG AAATTGCCGG TTTAATGTTA
      701  TCAGCAATTA TCTCAGATAG CTTACTTTTC AAATCACAAAC ATGTACACAA
      751  CAAGATGTTA AAGCAGCTGA AGAATTAAAA GATATTGCTA AAGTTGATAT
      801  TCAAAAGTAC GGCTTAGATA TGTTAAAAGC AGGTGCTTCA ACAACTGATA
45    851  AATCAGTTGA ATTCTTATTA AACATGGATG CTAAATCATT TACTATGGGT
      901  GACTATGKGA YTCGTATTGC AACAAAGTTAA TGCTGTTGAC CTTGACGAAG

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5      951  TGTAAWTCG TAAAGAAGAT TTAGAAAAAG AAATGTTAGC TGTAAGTGCA
      1001  CAAGAAAAAT ATGACTTATT TGTACTTGTT GTTACKGACA TCATTAATAG
      1051  TGATTCTAAA ATTTTAGTTG TAGGTGCTGA AAAAGATAAA GTTGGCGAAG
      1101  CATTCAATGT TCAATTAGAA GATGACATGG CCYTCTTATC TGGTGTCGTW
      1151  TCTCGAAAAA AACAAATCGT ACCTCAAATC ACTGAAGCAT TAACAAAATA
      1201  ATACTATATT ACTGTCTAAT TATAGACATG TTGTATTTAA CTAACAGTTC
      1251  ATTAAAGTAG AATTTATTTT ACTTTCCAAT GAACTGTTTT TTATTTACGT
      1301  TTGACTAATT TACAACCCTT TTTCAATAGT AGTTTTTTATT CCTTTAGCTA
      1351  CCTAACCCA CAGATTAGTG ATTTCTATAC AATTCCCCTT TTGTCTTAAC
10     1401  ATTTTCTTAA AATATTTGCG ATGTTGAGTA TAAATTTTTG TTTTCTTCCT
      1451  ACCTTTTTTCG TTATGATTAA AGTTATAAAT ATTATTATGT ACACGATTCA
      1501  TCGCTCTATT TTCAACTTTC AACATATATA ATTGAGAAAG CCATTTAAAA
      1551  TTAACGGCCA CAACATTCAA ATCAATTAAT CGCTTTTTTCC AAAATAATCA
      1601  TATAAGGAGG TTCTTTTCAT TATGAATATC ATTGAGCAAA AATTTTATGA
15     1651  CAGTAAAGCT TTTTTCATA CACAACAAAC TAAAGATATT AGTTTATGAA
      1701  AAGAGCAATT AAAGAAGTTA AGCAAAGCTA TTAAATCATA CGAGAGCGAT
      1751  ATTTTAGAAG CACTATATAC AGATTTAGGA AAAAATAAAG TCGAAGCTTA
      1801  TGCTACTGAA ATTGGCATAA CTTTGAAAAG TATCAAATTT GCCCGTAAGG
      1851  AACTTAAAAA CTGGACTAAA CAAAAAATG TAGACACACC TTTATATTTA
20     1901  TTTCCAACAA AAAGCTATAT CAAAAAAGAA CCTTATGGAA CAGTT

```

25 Mutant: NT 201

Phenotype: temperature sensitivity

Sequence map: Mutant NT201 is complemented by plasmid pMP269, which carries a 2.6 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 68. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level similarity to *ylxC*, encoding a putative *murB* homolog (UDP-N-acetylenolpyruvoylglucosamine reductase), in *B. subtilis* (Genbank Accession No. M31827). The predicted relative size and orientation of the *ylxC* gene is depicted by an arrow in the map.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP269, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP269

5 SEQ ID NO. 78

pMP269 Length: 2590 nt

```
1   TCGAACTCGG TACCCGGGGA TCCTCTAGAG TCGATCAACT ACAACTACAA
51  TTAAACAAAT TGAGGAACTT GATAAAGTTG TAAAATAATT TTAAAGAGG
10  101  GGAACAATGG TTAAAGGTCT TAATCATTGC TCCCCTCTTT TCTTTAAAAA
151  AGGAAATCTG GGACGTCAAT CAATGTCCTA GACTCTAAAA TGTTCTGTTG
201  TCAGTCGTTG GTTGAATGAA CATGTACTTG TAACAAGTTC ATTTCAATAC
251  TAGTGGGCTC CAAACATAGA GAAATTTGAT TTTCAATTTT TACTGACAAT
301  GCAAGTTGGC GGGGCCCAAA CATAGAGAAT TTCAAAAAGG AATTCTACAG
15  351  AAGTGGTGCT TTATCATGTC TGACCCACTC CCTATAATGT TTTGACTATG
401  TTGTTTAAAT TTCAAAATAA ATATGATAGT GATATTTACA GCGATTGTTA
451  AACCGAGATT GGCAATTTGG ACAACGCTCT ACCATCATAT ATTCAATTGAT
501  TGTTAATTCTG TGTTCGCATA CACCGCATAA GATTGCTTTT TCGTTAAATG
551  AAGGCTCAGA CCAACGCTTA ATGGCGTGCT TTTCAAATCT ATTATGGCAC
20  601  TTATAGCATG GATAGTATTT ATTACAACAT TTAAATTTAA TAGCAATAAT
651  ATCTTCTTCG GTAAAATAAT GGCGACAGCG TGTTTCAGTA TCGATTAATG
701  AACCATAAAC TTTAGGCATA GACAAAGCTC CTTAACTTAC GATTCCTTTG
751  GATGTTTACC AATAATGCGA ACTTCACGAT TTAATTCAAT GCCAAWTTTT
801  TCTTTGACGG TCTTTTGTAC ATAATGAATA AGGTTTTCAT AATCTGTAGC
25  851  AGTTCCATTG TCTACATTTA CCATAAAACC AGCGTGTGTT GTTGAACTT
901  CAACGCCGCC AATACGGTGA CCTTGCAAAT TAGAATCTTG TATCAATTTA
951  CCTGCAAAT  GACCAGGCGG TCTTTGGAAT ACACTACCAC ATGAAGGATA
1001 CTCTAAAGGT TGTTTAAATT CTCTACGTTT TGTTAAATCA TCCATTTTAG
1051 CTTGTATTTT AGTCATTTTA CCAGGAGCTA AAGTAAATGC AGCTTCTAAT
30  1101 ACAACTAANT GTTCTTTTTT AATAATGCTA TTACNATAAT CTAACTCTAA
1151 TTCTTTTGTT GTAAGTTTAA TTAACGAGCC TTGTTGTTTT ACGCAAAGCG
1201 CATRGCTAT  ACAATCTTTA ACTTCGCCAC CATAAGCGCC AGCATTCTA
1251 TACACTGCAC CACCAATTGA ACCTGGAATA CCACATGCAA ATTCAAGGCC
1301 AGTAAGTGCG TAATCACGAG CAACACGTGA GACATCAATA ATTGCAGCGC
35  1351 CGCTACCGGC TATTATCGCA TCATCAGATA CTTCCGATAT GATCTAGTGA
1401 TAATAAACTA ATTACAATAC CGCGAATACC ACCTTCACGG ATAATAATAT
1451 TTGAGCCATT TCCTAAATAT GTAACAGGAA TCTCATTTTG ATAGGCATAT
1501 TTAACAACCTG CTTGTACTTC TTCATTTTTA GTAGGGGTAA TGTAAAAGTC
1551 GGCATTACCA CCTGTTTTAG TATAAGTGTA TCGTTTTTAA GGTTTCATCA
40  1601 CTTTAATTTT TTCAKTYGRS MTRARKKSWT GYAAAGCTTG ATAGATGTCT
1651 TTATTTATCA CTTCTCAGTA CATCCTTTCT CATGTCTTTA ATATCATATA
1701 GTATTATACC AATTTTAAAA TTCATTTGCG AAAATTGAAA AGRAAGTATT
1751 AGAATTAGTA TAATTATAAA ATACGGCATT ATTGTCGTTA TAAGTATTTT
1801 TTACATAGTT TTTCAAAGTA TTGTTGCTTT TGCATCTCAT ATTGTCTAAT
45  1851 TGTTAAGCTA TGTGCAATA TTTGGTGTTT TTTTGTATTG AATTGCAAAG
1901 CAATATCATC ATTAGTTGAT AAGAGGTAAT CAAGTGCAAG ATAAGATTCA
1951 AATGTTTGGG TATTCATTTG AATGATATGT AGACGCACCT GTTGTTTTTAG
2001 TTCATGAAAA TTGTTAAACT TCGCCATCAT AACTTTCTTA GTATATTTAT
2051 GATGCAAACG ATAAAACCCCT ACATAATTTA AGCGTTTTTC ATCTAAGGAT
50  2101 GTAATATCAT GCAAATTTTC TACACCTACT AAAATATCTA AAATTGGCTC
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2151 TGTTGAATAT TTAAATGAT GCGTACCGCC AATATGTTTT GTATATTTTA
 2201 CTGGGCTGTC TAAGAGGTTG AATAATAATG ATTCAATTTT AGTGTATTGT
 2251 GATTGAAAAC AATTAGTTAA ATCACTATTA ATGAATGGTT GAACATTTGA
 5 2301 ATACATGATA AACTCCTTTG ATATTGAAAA TTAATTTAAT CACGATAAAG
 2351 TCTGGAATAC TATAACATAA TTCATTTTCA TAATAAACAT GTTTTTGTAT
 2401 AATGAATCTG TTAAGGAGTG CAATCATGAA AAAAATTGTT ATTATCGCTG
 2451 TTTTAGCGAT TTTATTTGTA GTAATAAGTG CTTGTGGTAA TAAAGAAAAA
 2501 GAGGCACAAC ATCMATTTAC TAAGCAATT AAAGATGTTG AGCAAACACA
 10 2551 WAAAGAATTA CAACATGTCA TGGATAATAT ACATTTGAAA

Mutant: NT304

15 **Phenotype:** temperature sensitivity

Sequence map: Mutant NT304 is complemented by plasmid
 pMP450, which carries a 3.3 kb insert of wild-type *S.*
aureus genomic DNA. A partial restriction map is depicted
 in Fig. 69. Database searches at the nucleic acid and
 20 (putative) polypeptide levels against currently available
 databases reveal strong peptide-level similarities from the
 left-most contig below and the *dod* gene product, encoding
 pentose-5-phosphate epimerase (EC 5.1.3.1), from *S.*
oleraceae (Genbank Accession No. L42328).

25 **DNA sequence data:** The following DNA sequence data
 represents the sequence generated from clone pMP450,
 starting with standard M13 forward and M13 reverse
 sequencing primers; the sequence contig will be completed
 30 via primer walking strategies. The sequence below can be
 used to design PCR primers for the purpose of amplification
 from genomic DNA with subsequent DNA sequencing.

clone pMP450

35

SEQ ID NO. 79

pMP450.forward Length: 1019 nt

1 ATTCGAGCTC GGTACCCGGG GATCCTCTAG AGTCGCTCGA TAACTTCTAT
 40 51 ATGAACATCA TGTTTATAAT ATGCTTTTTT CAATAATAAC TGAATTGCCC
 101 CAAAAAAGTG ATCTAATCGT CCGCCTGTTG CACCATAAAT TGTAATACTA
 151 TCAAATCCAA GTGCAACAGC TTTATCAACC GCTAAAGCTA AATCCGTATC
 201 AGCTTTTTCA GCTTGAACTG GTTTGATTG TAACTGTTCT GTTAGAAGTT
 251 GGC GTTCTTC TTTACTGACT GAATCAAAGT CTCCCACTGA GAAAAAAGGG
 45 301 ATAATTTGAT GCTTCAATAA AATCAAAGCA CCTCTATCAA CGCCGCCCCA
 351 TTTACCTTCA TTACTTTTGG CCCAAATATC TTGCGGCAAG TGTCGATCAG

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5  401 AACATAATAA ATTTATATGC ATATACACTC AACCTTTCAA TGCTTGTGTT
    451 GACTTTTTTA TAATCCTCTT GTTTAAAGAA AAATGAACCT GTTACTAGCA
    501 TTGTTAGCAC CATTTTCAAC ACAAACTTTC GCTGTTATCG GTATTTACGC
    551 CTCCATCAAC TTCAATATCA AAGTTTAATT GACGTTCCAT TTTAATAGCA
    601 TTAAGACCCG CTATTTTTTC TACGCATTGA TCAATAAATG ATTGACCACC
    651 AAACCCTGGG TTAAGTGTCA TCACTAGTAC ATAATCAACA ATGTCTAAAA
    701 TAGGTTCAAT TTGTGATATT GGTGTACCAG GATTAATTAC TACACCAGCT
    751 TTTTATCTA AATGTTTAAT CATTGAATA GCACGATGAA ATATGAGGCG
10  801 TTGATTCGAC ATGAATTGNA AATCATATCG GCACCATGTT CTGCAAATGA
    851 TGCAATATAC TTTTCTGGAA TTTTCAATCA TCAAATGTAC GTCTATANGT
    901 AATGTTGTGC CTTTCTTAC TGCATCTAAT ATTGGTAAAC CAATAGATAT
    951 ATTAGGGACA AATTGACCAT CCATAACATC AAAATGAACT CCGTCGAANC
   1001 CCGGCTTCTC CAGTCGTTT

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15 SEQ ID NO. 80

pMP450.reverse Length: 1105 nt

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    1 CNTGCATGCC TGCAGGTCGA TCTANCAAAG CATATTAGTG AACATAAGTC
   51 GAATCAACCT AAACGTGAAA CGACGCAAGT ACCTATTGTA AATGGGCCTG
20 101 CTCATCATCA GCAATTCCAA AAGCCAGAAG GTACGGTGTA CGAACCAAAA
   151 CCTAAAAAGA AATCAACACG AAAGATTGTG CTCTTATCAC TAATCTTTTC
   201 GTTGTTAATG ATTGCACTTG TTTCTTTTGT GGCAATGGCA ATGTTTGGTA
   251 ATAAATACGA AGAGACACCT GATGTAATCG GGAAATCTGT AAAAGAAGCA
   301 GAGCAAATAT TCAATAAAAA CAACCTGAAA TTGGGTAAAA TTTCTAGAAG
25 351 TTATAGTGAT AAATATCCTG AAAATGAAAT TATTAAGACA ACTCCTAATA
   401 CTGGTGAACG TGTGGAACGT GGTGACAGTG TTGATGTTGT TATATCAAAG
   451 GGSCCTGAAA AGGTAAAAAT GCCAAATGTC ATTGGTTTAC CTAAGGAGGA
   501 AGCCTTGACG AAATTAATAA CCGTTAGGTC TTAAAGATGT TACGATTGAA
   551 AAAGTWTATA ATAATCCAAG CGCCMAAAGG ATACATTGCA AATCAAATGT
30 601 TTAMCCGCAA ATACTGAAAT CGCTATTCAT GATTCTAATA TTAACTATA
   651 TGAATCTTTA GGCATTAAGC AAGTTTATGT AGAAGACTTT GAACATAAAT
   701 CCTTTAGCAA AGCTAAAAAA GCCTTAGAAG AAAAAGGGTT TAAAGTTGAA
   751 AGTAAGGAAG AGTATAGTGA CGATATTGAT GAGGGTGATG TGATTCTCTA
   801 ATCTCCTAAA GGAAAAATCAG TAGATGAGGG GTCAACGATT TCATTGTTG
35 851 TTTCTAAAGG TAAAAAAGT GACTCATCAG ATGTCNAAAC GACAACTGAA
   901 TCGGTAGATG TTCCATACAC TGGTNAAAAT GATAAGTCAC AAAAAGTTCT
   951 GGTTTATCTT NAAGATAANG ATAATGACGG TTCCACTGAA AAAGGTAGTT
  1001 TCGATATTAC TAATGATCAC GTTATAGACA TCCTTTAAGA ATTGAAAAAG
  1051 GGAAAACGCA GTTTTATTGT TAAATTGACG GTAAACTGTA CTGAAAAAAA
40 1101 NTCGC

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45 Mutant: NT 310

Phenotype: temperature sensitivity

Sequence map: Mutant NT310 is complemented by plasmid pMP364, which carries a 2.4 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted

in Fig. 70; there are no apparent restriction sites for EcoR I, BamH I, Hind III or Pst I. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong similarities to the *ddlA* gene product from *E. hirae*, which encodes D-Ala-D-Ala ligase (EC 6.3.2.4); similarities are also noted to the functionally-similar proteins VanA and VanB from *E. faecium* and the VanC protein from *E. gallinarum*. The predicted relative size and orientation of the *ddlA* gene is depicted by an arrow in the restriction map.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP364, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP364

SEQ ID NO. 81

pMP364 Length: 2375 nt

```

25      1  AATATGACAG AACCGATAAA GCCAAGTTCC TCTCCAATCA CTGAAAAGAT
      51  AAAGTCAGTA TGATTTTTCAG GTATATAAAC TTCACCGTGA TTGTATCCTT
     101  TACCTAGTAA CTGTCCAGAA CCGATAGCTT TAAGTGATTG AGTTAAATGA
     151  TAGCCATCAC CACTACTATA TGTATAGGGG TCAAGCCATG AATTGATTGG
     201  TCCCATTTGA TACAGTTGGA CACCTAATAA ATTTTCAATT AATGCGGGTG
     251  CATATAGAAT ACCTAAAATG ACTGTCATTG CACCAACAAT ACCTGTAATA
     301  AAGATAGGTG CTAAGATACG CCATGTTATA CCACTTACTA ACATCACACC
     351  TGCAATAATA GCAGCTAATA CTAATGTAGT TCCTAGGTCA TTTTGCAGTA
     401  ATATTAAAAT ACTTGGTACT AACGAGACAC CAATAATTTT GAAAAATAAT
     451  AACAAATCAC TTTGGAATGA TTTATTGAAT GTGAATTGAT TATGTCTAGA
     501  AACGACACGC GCTAATGCTA AAATTAAAAT AATTTTCATG AATTCAGATG
     551  GCTGAATACT GATAGGGCCA AACGTGTTYC AACTTTTGGC ACCATTGATA
     601  ATAGGTGTTA TAGGTGACTC AGGAATAACG AACCAGCCTA TTWATAWTAG
     651  ACAGATTAAAG AAATACAATA AATATGTATA ATGTTTAAATC TTTTtaggtg
     701  AAATAAACAT GATGATACCT GCAAAAATTG CACCTAAAAT GTAATAAAAA
     751  ATTTGTCTGA TACCGAAATT AGCACTGTAT TGACCACCGC CCATTGCCGA
     801  GTTAATAAGC AGAACACTGA AAATTGCTAA AACAGCTATA GTGGCTACTA
     851  ATACCCAGTC TACTTTGCGA AGCCAATGCT TATCCGGCTG TTGACGAGAT
     901  GAATAATTCA TTGCAAACTC CTTTtataCT CACTAATGTT TATATCAATT
     951  TTACATGACT TTTTAAAAAT TAGCTAGAAT ATCACAGTGA TATCAGCYAT
    1001  AGATTTCAAT TTGAATTAGG AATAAAAATAG AAGGGAATAT TGTCTGATT
    1051  ATAAATGAAT CAACATAGAT ACAGACACAT AAGTCCTCGT TTTTAAAAAT
  
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1101 CAAAATAGCA TTAAATGTG ATACTATTAA GATTCAAAGA TGCGAATAAA
1151 TCAATTAACA ATAGGACTAA ATCAATATTA ATTTATATTA AGGTAGCAAA
1201 CCCTGATATA TCATTGGAGG GAAAACGAAA TGACAAAAGA AAATATTTGT
1251 ATCGTTTTTG GAGGGAAAAG TGCAGAACAC GAAGTATCGA TTCTGACAGC
5 1301 AYWAAATGTA TTAAATGTCAR TAGATAAAGA CAAATATCAT GTTGATATCA
1351 TTTATATTAC CAATGATGGT GATTGGAGAA AGCAAAATAA TATTACAGCT
1401 GAAATTAAAT CTACTGATGA GCTTCATTTA GAAAAATGGA GAGGCGCTTG
1451 AGATTTTACA GCTATTGAAA GAAAGTAGTT CAGGACAACC ATACGATGCA
1501 GTATTCCCAT TATTACATGG TCCTAATGGT GAAGATGGCA CGATTCAAGG
10 1551 GCTTTTTGAA GTTTTGGATG TACCATATGT AGGAAATGGT GTATTGTCAG
1601 CTGCAAGTTT CTATGGACAA ACTTGTAATG AAACAATTAT TTGAACATCG
1651 AGGGTTACCA CAGTTACCTT ATATTAGTTT CTTACGTTCT GAATATGAAA
1701 AATATGAACA TAACATTTTA AAATTAGTAA ATGATAAATT AAATTACCCA
1751 GTCTTTGTTA AACCTGCTAA CTTAGGGTCA AGTGTAGGTA TCAGTAAATG
15 1801 TAATAATGAA GCGGAACCTTA AAGGAGGTAT TAAAGAAGCA TTCCAATTTG
1851 ACCGTAAGCT TGTATAGAA CAAGGCGTTA ACGCAACGTG AAATTGAAGT
1901 AGCAGTTTTA GGAAATGACT ATCCTGAAGC GACATGGCCA GGTGAAGTCG
1951 TAAAAGATGT CGCGTTTTAC GATTACAAAT CAAAATATAA AGGATGGTAA
2001 GGTTCAATTA CAAATTCCAG CTGACTTAGA CGGAAGATGT TCAATTAACG
20 2051 GCTTAGAAAT ATGGCATTAG AGGCATTCAA AGCGACAGAT TGTTCTGGTT
2101 TAGTCCGTGC TGATTTCTTT GTAACAGAAG ACAACCAAAT ATATATTAAT
2151 GAAACAAATG CAATGCCTGG ATTTACGGCT TTCAGTATGT ATCCAAAGTT
2201 ATGGGAAAAT ATGGGCTTAT CTTATCCAGA ATTGATTACA AAACCTATCG
2251 AGCTTGCTAA AGAACGTCAC CAGGATAAAC AGAAAAATAA ATACAAATT
25 2301 SMCTWAMTGA GGTTGTTATK RTGATTAAYG TKACMYTAWA GYAAAWTCAA
2351 TCATGGATTN CCTTGTGAAA TTGAA

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30

Mutant: NT 312

Phenotype: temperature sensitivity

Sequence map: Mutant NT312 is complemented by plasmid pMP266, which carries a 1.5 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 71; there are no apparent restriction sites for EcoR I, BamH I, Hind III or Pst I. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level similarities to *mg442*, a hypothetical ORF from *M. genetium*, and limited similarities to G-proteins from human and rat clones; this probably indicates a functional domain of a new Staph. protein involved in GTP-binding. The ORF contained within clone pMP266 is novel and likely to be a good candidate for screen development.

45

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP266, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP266

SEQ ID NO. 82

pMP266 Length: 1543 nt

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15      1 AATCATTTTC AGTTTATCAT TAAACAAATA TATTGAACYM MYMAAAATGT
      51 CATACTGATA AAGATGAATG TCACTTAATA AGTAACTTAG ATTTAACAAA
     101 TGATGATTTT TAATTGTAGA AAACCTGAAA TAATCACTTA TACCTAAATC
     151 TAAAGCATTG TTAAGAAGTG TGACAATGTT AAAATAAATA TAGTTGAATT
     201 AATGAATTTG TTCTAYAATT AACAKGTTWT WGAWTTTAAT AATGAGAAAA
20      251 GAATTGACGA AAGTAAGGTG AATTGAATGG TTATTCMATG GTATCCAGGA
     301 CMTATGGCGA AAAGCCAAAA GAGAAGTAAG TGAACAATTA AMAAAAGTAG
     351 ATGTAGTGTT TGAAGTAGTA GATGCAAGAA TTCCATATAG TTCAAGAAAC
     401 CCTATGATAG ATGAAGTTAT TAACCAAAAA CCACGTGTTG TTATATTAAA
     451 TAAAAAAGAT ATGTCTAATT TAAATGAGAT GTCAAAATGG GAACAATTTT
25      501 TTATTGATAA AGGATACTAT CCTGTATCAG TGGATGCTAA GCACGGTAAA
     551 AATTTAAAGA AAGTGGAAGC TGCAGCAATT AAGGCGACTG CTGAAAAATT
     601 TGAACGCGAA AAAGCGAAAG GACTTAAACC TAGAGCGATA AGAGCAATGA
     651 TCGTTGGAAT TCCAAATGTT GGTAAATCCA CATTAATAAA TAACTGGCA
     701 AAGCGTAGTA TTGCGCAGAC TGGTAATAAA CCAGGTGTGA CCAAACAACA
30      751 ACAATGGATT AAAGTTGGTA ATGCATTACA ACTATTAGAC ACACCAGGGA
     801 TACTTTGGCC TAAATTTGAA GATGAAGAAG TCGGTAAGAA GTTGAGTTTA
     851 ACTGGTGCGA TAAAAGATAG TATTGTGCAC TTAGATGAAG TTGCCATCTA
     901 TGGATTAAAC TTTTAAATTC AAAATGATTT AGCGCGATTA AAGTCACATT
     951 ATAATATTGA AGTTCCTGAA GATGCMGAAA TCATAGCGTG GTTTGATGCG
35     1001 ATAGGGAAAA AACGTGGCTT AATTCGACGT GGTAAATGAAA TTGATTACGA
     1051 AGCAGTCATT GAACTGATTA TTTATGATAT TCGAAATGCT AAAATAGGAA
     1101 ATTATTGTTT TGATATTTTT AAAGATATGA CTGAGGAATT AGCAAATGAC
     1151 GCTAACAATT AAAGAAGTTA CGCAGTTGAT TAATGCGGTT AATACAATAG
     1201 AAGAATTAGA AAATCATGAA TGCTTTTTAG ATGAGCGAAA AGGTGTTCAA
40     1251 AATGCCATAG CTAGGCGCAG AAAAGCGTTA GAAAAAGAAC AAGCTTTAAA
     1301 AGAAAAGTAT GTTGAAATGA CTTACTTTGA AAATGAAATA TTAAAAGAGC
     1351 ATCCTAATGC TATTATTTGT GGGATTGATG AAGTTGGAAG AGGACCTTTA
     1401 GCAGGTCCAG TCGTTGCATG CGCAACAATT TTAAATTCAA ATCACAATTA
     1451 TTTGGGCCTT GATGACTCGA AAAAAGTACC TGTTACGAAA CGTCTAGAAT
45     1501 TAAATGAAGC ACTAAAAAAT GAAGTTACTG YTTTTGCATA TGG

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Mutant: NT 318

Phenotype: temperature sensitivity

Sequence map: Mutant NT318 is complemented by plasmid
 5 pMP270, which carries a 2.2 kb insert of wild-type *S.*
aureus genomic DNA. A partial restriction map is depicted
 in Fig. 72; there are no apparent restriction sites for
 EcoR I, BamH I, Hind III, or Pst I. Database searches at
 the nucleic acid and (putative) polypeptide levels against
 10 currently available databases reveal strong similarities to
 the *spoVC* gene from *B. subtilis*, a gene identified as being
 important in sporulation, and the *pth* gene from *E. coli*,
 which encodes aminoacyl-tRNA hydrolase (EC 3.1.1.29). It
 is highly likely that the *spoVC* and *pth* gene products are
 15 homologues and that the essential gene identified here is
 the Staph. equivalent. The predicted relative size and
 orientation of the *spoVC* gene is depicted by an arrow in
 the restriction map.

20 **DNA sequence data:** The following DNA sequence data
 represents the sequence generated by primer walking through
 clone pMP270, starting with standard M13 forward and M13
 reverse sequencing primers and completing the sequence
 contig via primer walking strategies. The sequence below
 25 can be used to design PCR primers for the purpose of
 amplification from genomic DNA with subsequent DNA
 sequencing.

clone pMP270

30

SEQ ID NO. 83

pMP270 Length: 2185 nt

	1	TTAAACAATT	AAGAAAATCT	GGTAAAGTAC	CAGCASYAGT	ATACGGTTAC
35	51	GGTACTAAAA	ACGTGTCAGT	TAAAGTTGAT	GAAGTAGAAT	TCATCAAAGT
	101	TATCCGTGAA	GTAGGTCGTA	ACGGTGTTAT	CGAATTAGGC	GTTGGTTCTA
	151	AAACTATCAA	AGTTATGGTT	GCAGACTACC	AATTCGATCC	ACTTAAAAAC
	201	CAAATTACTC	ACATTGACTT	CTTWKCAATC	AATATGAGTG	AAGAACGTAC
	251	TGTTGAAGTA	CCAGTTCAAT	TAGTTGGTGA	AGCAGTAGGC	GCTAAAGAAA
40	301	GGCGGCGTTA	GTTGAACAAC	CATTATTCAA	CTTAGAAAAGT	AACTGCTACT
	351	CCAGACAATA	TTCCAGAAGC	AATCGAAGTA	GACATTACTG	AATTAAACAT
	401	TAACGACAGC	TTAACTGTTG	CTGATGTTAA	AGTAACTGGC	GACTTCAAAA
	451	TCGAAAACGA	TTCAGCTGAA	TCAGTAGTAA	CAGTAGTTGC	TCCAAC TGAA
	501	GAACCAACTG	AAGAAGAAAT	CGAAGCCTAT	GGAAGGCGAA	CAMCAAACTG
45	551	AAGAACCAGA	AGTTGTTGGC	GAAAGCAAAG	AAGACGAAGA	AAAAACTGAA

5 601 GAGTAATTTT AATCTGTTAC ATTAAAGTTT TTATACTTTG TTTAACAAGC
651 ACTGTGCTTA TTTTAATATA AGCATGGTGC TTTTKGTGTT ATTATAAAGC
701 TTAATTAAAC TTTATWACTT TGTACTAAAG TTTAATTAAT TTTAGTGAGT
751 AAAAGACATT AAACCTCAACA ATGATACATC ATAAAAATTT TAATGTACTC
801 GATTTTAAAA TACATACTTA CTAAGCTAAA GAATAATGAT AATTGATGGC
851 AATGGCGGAA AATGGATGTT GTCATTATAA TAATAAATGA AACAAATTATG
901 TTGGAGGTAA ACACGCATGA AATGTATTGT AGGTCTAGGT AATATAGGTA
951 AACGTTTTGA ACTTACAAGA CATAATATCG GCTTTGAAGT CGTTGATTAT
10 1001 ATTTTAGAGA AAAATAATTT TTCATTAGAT AAACAAAAGT TTAAAGGTGC
1051 ATATACAATT GAACGAATGA ACGGCGATAA AGTGTTATTT ATCGAACCAA
1101 TGACAATGAT GAATTTGTCA GGTGAAGCAG TTGCACCGAT TATGGATTAT
1151 TACAATGTTA ATCCAGAAGA TTTAATTGTC TTATATGATG ATTTAGATTT
1201 AGAACAAGGA CAAGTTCGCT TAAGACAAAA AGGAAGTGCG GCGCGTCACA
1251 ATGGTATGAA ATCAATTATT AAAATGCTTG GTACAGACCA ATTTAAACGT
15 1301 ATTCGTATTG GTGTGGGAAG ACCAACGAAT GGTATGACGG TACCTGATTA
1351 TGTTTTACAA CGCTTTTCAA ATGATGAAAT GGTAACGATG GGAAAAAGTT
1401 ATCGAACACG CAGCACGCGC AATTGAAAAG TTTGTTGAAA CATCACRATT
1451 TGACCATGTT ATGAATGAAT TTAATGGTGA AKTGAAATAA TGACAATATT
1501 GACAMCSCTT ATAAAAGAAG ATAATCATTT TCAAGACCTT AATCAGGTAT
20 1551 TTGGACAAGC AAACACACTA GTAACGGTGC TTTCCCCGTC AGCTAAAGTG
1601 ACGATGATTG CTGAAAAATA TGCACAAAGT AATCAACAGT TATTATTAAT
1651 TACCAATAAT TTATACCAAG CAGATAAATT AGAAACAGAT TTAATTCAAT
1701 TTATAGATGC TGAAGAATTG TATAAGTATC CTGTGCAAGA TATTATGACC
1751 GAAGAGTTTT CAACACAAAG CCCTCAACTG ATGAGTGAAC GTATTAGAAC
25 1801 TTTAACTGCG TTAGCTCCAA GGTAAGAAAG GGTTATTTAT CGTTCCTTTA
1851 AATGGTTTGA AAAAGTGGTT AACTCCTGTT GAAATGTGGC AAAATCACCA
1901 AATGACATTG CGTGTTGGTG AGGATATCGA TGTGGACCAA TTTMWWAACA
1951 AATTAGTTAA TATGGGGTAC AAACGGGAAT CCGTGGTATC GCATATTGGT
2001 GAATTCTCAT TGCGAGGAGG TATTATCGAT ATCTTTCCGC TAATTGGGGA
30 2051 ACCAATCAGA ATTGAGCTAT TTGATACCGA AATTGATTCT ATTCGGGATT
2101 TTGATGTTGA AACGCAGCGT TCCAAAAGATA ATGTTGAAGA AGTCGATATC
2151 ACAACTGCAA GTGATTATAT CATTACTGAA GAAGT

35

Mutant: NT 321

Phenotype: temperature sensitivity

Sequence map: Mutant NT321 is complemented by plasmid
40 pMP276, which carries a 2.5 kb insert of wild-type *S.*
aureus genomic DNA. A partial restriction map is depicted
in Fig. 73; no apparent sites for Hind III, EcoR I, BamH I
or Pst I are present. Database searches at the nucleic
acid and (putative) polypeptide levels against currently
45 available databases reveal strong peptide-level
similarities to a hypothetical ORF of unknown function from
M. tuberculosis (Genbank Accession No. Z73902).

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP276, starting with standard M13 forward and M13 reverse sequencing primers, and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

10 clone pMP276

SEQ ID NO. 84

pMP276 Length: 2525 nt

```
15      1  AATCTGTTCC TACTACAATA CCTTGTCGGT TTGAAGCACC NGAAAATNGT
      51  ACTTTCATAC GTTCACGCGC TTTTTCATTT CCTTTTGGGA AATCTGTAAG
    101  AACAAATACCG GCTTCTTTTA ATGATTGCAC ACTTTGATCA ACTGCAGGCT
    151  TAATATTGAC TGTTACTATT TCATCTGGTT CAATGAATCG CAAAGCTTGC
    201  TCAACTTCAT CAGCATCTTT TTGAACTCCA TAAGGTAATT TAACTGCAAT
    20      251  AAACGTACAA TCAATGCCTT CTTACGTAA TTCGTTAACA GACATTTGTA
    301  CTAGTTTTCC AACTAATGTA GAATCCTGTC CTCCTGAAAT ACCTAACACT
    351  AAAGATTTTA TAAATGAATG TGATTGTACA TAATTTTTTA TAAATGCTT
    401  TAATTCCATA ATTTCTTCAG CACTATCGAT ACGCTTTTTT ACTTTCATTT
    451  CTTGTACAAT AACGTCTTGT AATTTACTCA TTATCTTCTT CCATCTCCTT
    25      501  AACGTGTTCC GCAACTTCAA AAATACGTTT ATGTTTATTA TCCCAACATG
    551  CCTTGCTTAA ATCGACTGGA TATTCTTG TGATTGAGAA ACGCTTATTT
    601  TCATCCCAAA TAGATTGTAA TCCTAGTGCT AAATATTCAC GTGATTTCATC
    651  TTCTGTTGGC ATTTGATATA CTAATTTACC ATTTTCATAA ATATTATGAT
    701  GCAAATCAAT GGCTTCGAAA GATTTTATAA ATTTTCATTT ATAAGTATGC
    30      751  ACTGGATGGA ATAATTTTAA AGGTTGTTCA TCGTATGGAT TTTTCATTTT
    801  CAAAGTAATA TAATCGCCTT CTGCCTTACC TGTTTTCTTG TTTATAATGC
    851  GATATACATT TTTCTTACCT GCGCTCGTAA CCTTTTCAGC GTTATTTGAT
    901  AATTTAATAC GATCACTATA TGAACCATCT TCATTTTCAA TAGCTACAAG
    951  TTTATATACT GCACCTAATG CTGGTTGATC GTATCCTGTA ATCAGCTTTG
    35      1001  TACCAACGCC CCAAGAATCT ACTTTTGCAC CTTGTGCTTT CAAACTCGTA
    1051  TTCGTTTCTT CATCCAAATC ATTAGAYGCG ATAATTTTAG TTTTCAGTAAA
    1101  TCCTGYTTCA TCAAGCATAC GTCTTGCTC TTTAGATAAA TAAGCGATAT
    1151  CTCCAGAATC TAATCGAATA CCTAACAAAG TTAATTTTGT CACCTAATTC
    1201  TTTTGCAACT TTTATTGCAT TTGGCACGCC AGATTTTAAA GTATGGAATG
    40      1251  TATCTACTAG GAACACACAA TTTTATGTC TTTTCAGCATA TTTTGTGAAG
    1301  GCAACATATT CGTCTCCATA AGTTTGGACA AATGCATGTG CATGTGTACC
    1351  AGACACAGGT ATACCAAATA ATTTTCCCCG CCCTAACATT ACTTGTAGAA
    1401  TCAAAGCCCC CGATGTAAGC AGCTCTAGCG CCCCACAATG CTGCATCAAT
    1451  TTCTTGCGCA CGACGTGTTA CCAAACCTCA TTAATTTATC ATTTGATGCA
    45      1501  ATTTGACGAA ATTCTGCTAG CCTTTGTTGT AATTAATGTA TGGAAATTTA
    1551  CAATGTTTAA TAAAATTGTT CTATTAATTG CGCTTGAATC AATGGTGCTT
    1601  CTACGCGTAA CAATGGTTCG TTACCAAAGC ATAATTCGCC TTCTTGATC
    1651  GAACGGATGC TGCCTGTGAA TTTTAAATCT TTTAAATATG ATAAGAAATC
    1701  ATCCTTGTAG CCAATAGACT TTAAATATTC CAAATCAGAT TCTGAAAATC
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1751 CAAAATGTTT TATAAAATCA ATGACGCGTT TTAAACCATT AAAAACAGCA
1801 TAGCCACTAT TAAATGGCAT TTTTCTAAAA TACAAATCAA ATACAGCCAT
1851 TTTTTCATGA ATATTATCAT TCCAATAACT TTCAGCCATA TTTATTGAT
1901 ATAAGTCATT ATGTAACATT AAAGTGTCTG CTTCTAATTG GTACACTTGT
5 1951 ATCTCTCCAA TCGACCTAAA TATTTTCTTA CATTTTATCA TAATTCATTT
2001 TTTTATATAC ATAAGAGCCC CTTAATTTCC ATACTTTTAA TTAAAAACAA
2051 CCAACAATTT AATGACATAT ACATAATTTT TAAGAGTATT TTAATAATGT
2101 AGACTATAAT ATAAAGCGAG GTGTTGTTAA TGTTATTTAA AGAGGCTCAA
2151 GCTTTCATAG AAAACATGTA TAAAGAGTGT CATTATGAAA CGCAAATTAT
10 2201 CAATAAACGT TTACATGACA TTGAACTAGA AATAAAAGAA ACTGGGACAT
2251 ATACACATAC AGAAGAAGAA CTTATTTATG GTGCTAAAT GGCTTGCGCT
2301 AATTCAAATC GTTGCAATTG TCGTTTATTT TGGGATTCGT TAAATGTCAT
2351 TGATGCAAGA GATGTTACTG ACGAAGCATC GTTCTTATCA TCAATTACTT
2401 ATCATATTAC ACAGGCTACA AATGAAGGTA AATTAAAGCC GTATATTACT
15 2451 ATATATGCTC CAAAGGATGG ACCTAAAATT TTCAACAATC AATTAATTCG
2501 CTATGCTGGC TATGACAATT GTGGT

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20

Mutant: NT 325

Phenotype: temperature sensitivity

Sequence map: Mutant NT325 is complemented by plasmid pMP644, which carries a 2.1 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 74; no apparent sites for *Hind* III, *Eco* R I, *Bam* H I or *Pst* I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal significant peptide-level similarities to the *ribC* gene product, a protein exhibiting regulatory functions, from *B. subtilis* (Genbank Accession No. x95312; unpublished).

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP644, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP644

45 SEQ ID NO. 85
pMP644 Length: 2181 nt

1 ATCGATAGGA AGAAGTACAA CGACTGAAGA TCAAACGGGT GATACATTGG
 51 AAACAAAAGG TGTACACTCA GCAGATTTTA ATAAGGACGA TATTGACCGA
 101 TTGTTAGAAA GTTTTAAAGG TATCATTGAA CAAATTCCGC CGATGTACTC
 5 151 ATCCGTCAAA GTAAATGGTA AAAAATTATA TGAATATGCG CGTAATAATG
 201 AAACAGTTGA AAGACCAAAG CGTAAAGTTA ATATTAAAGA CATTGGGCGT
 251 ATATCTGAAT TAGATTTTAA AGAAAAATGAG TGTCATTTTA AAATACGCGT
 301 CATCTGTGGT AAAGGTACAT ATATTAGAAC GCTAGCAACT GATATTGGTG
 351 TGAAATTAGG CTTTCCGGCA CATATGTCGA AATTAACACG AATCGAGTCT
 10 401 GGTGGATTTG TGTTGAAAGA TAGCCTTACA TTAGAACAAA TAAAAGAAGT
 451 TCATGAGCAG GATTCATTGC AAAATAAATT GTTTCCTTTA GAATATGGAT
 501 TAAAGGGTTT GCCAAGCATT AAAATTAAAG ATTCGCACAT AAAAAACGT
 551 ATTTTAAATG GGCAGAAATT TAATAAAAAT GAATTTGATA ACAAATTA
 601 AGACCAAATT GTATTTATTG ATGATGATTC AGAAAAAGTA TTAGCAATTT
 15 651 ATATGGTACA CCCTACGAAA AGAATCAGAA ATTAAACCTA AAAAAGTCTT
 701 TAATTAAAGG AGATAGAATT TATGAAAGTT CATAGAAAGT GACACATCCT
 751 ATACAATCCT AACAGTTAT ATTACAGGAG GATGTTGCAA TGGGCATTCC
 801 GGATTTTTTCG ATGGCATGCA TAAAGGTCAT GACAAAGTCT TTGATATATT
 851 AAACGAAATA GCTGAGGCAC GCAGTTTAAA AAAAGCGGTG ATGACATTTG
 20 901 ATCCGCATCC GTCTGTCGTG TTTGAATCCT AAAAGAAAAC GAACACGTTT
 951 TTACGCCCCT TTCAGATAAA ATCCGAAAAA TTACCCACAT GATATTGATT
 1001 ATTGTATAGT GGTTAATTTT TCATCTAGGT TTGCTAAAGT GAGCGTAGAA
 1051 GATTTTGTTG AAAATTATAT AATTAATAAT AATGTAAAG AAGTCATTGC
 1101 TGGTTTTGAT TTTAACTTTT GGTAAATTTG GAAAAGGTAA TATGACTGTA
 25 1151 ACTTCAAGAA TATGATGCGT TTAATACGAC AATTGTGAGT AAACAAGAAA
 1201 TTGAAAATGA AAAAATTTCT ACAACTTCTA TTCGTCAAGG ATTTAATCAA
 1251 TGGTGAGTTG CCAAAAAGGC GAATGGATGG CTTTTAGGCT ATATATATTT
 1301 CTTATTAAAA GGCAGTGTAG TGCAAGGTGA AAAAAGGGGA AGAAGTATTG
 1351 GCTTCCCCAA CAGCTAACAT TCAACCTAGT GATGATTATT TGTTACCTCG
 30 1401 TAAAGGTGTT TATGCTGTTA GTATTGAAAT CGGCACTGAA AATAAATTAT
 1451 ATCGAGGGGT AGCTAACATA GGTGTAAAGC CAACATTTCA TGATCCTAAC
 1501 AAAGCAGAAG TTGTCATCGA AGTGAATATC TTTGACTTTG AGGATAATAT
 1551 TTATGGTGAA CGAGTGACCG TGAATTGGCA TCATTTCTTA CGTCCTGAGA
 1601 TTAAATTTGA TGGTATCGAC CCATTAGTTA AACAAATGAA CGATGATAAA
 35 1651 TCGCGTGCTA AATATTTATT AGCAGTTGAT TTTGGTGATG AAGTAGCTTA
 1701 TAATATCTAG AGTTGCGTAT AGTTATATAA ACAATCTATA CCACACCTTT
 1751 TTTCTTAGTA GGTGCAATCT CCAACGCCTA ACTCGGATTA AGGAGTATTC
 1801 AAACATTTTA AGGAGGAAAT TGATTATGGC AATTTCAAA GAACGTAAAA
 1851 ACGAAATCAT TAAAGAATAC CGTGACACG AAAGTATAC TGGTTCACCA
 40 1901 GAAGTACAAA TCGCTGTACT TACTGCAGAA ATCAACGCAG TAAACGAACA
 1951 CTTACGTACA CACAAAAAAG ACCACCATTC ACGTCGTGGA TTATTAAAA
 2001 TGGTAGGTCG TCGTAGACAT TTATTAAACT ACTTACGTAG TAAAGATATT
 2051 CAACGTTACC GTGAATTAAT TAAATCACTT GGTATCCGTC GTTAATCTTA
 2101 ATATAACGTC TTTGAGGTTG GGGCATATTT ATGTTCCAAC CCTTAATTTA
 45 2151 TATTAAAAAA GCTTTTTTCA WRYMTKMASR T

Ph notype: temperature sensitivity

Sequence map: Mutant NT333 is complemented by plasmid pMP344, which carries a 2.3 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 75; no apparent restriction sites for EcoR I, Hind III, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal significant similarities to the *murD* gene product from *B. subtilis*, which encodes udp-MurNAc-dipeptide::D-Glu ligase (EC 6.3.2.9); similarities are also noted to the equivalent gene products from *E. coli* and *H. influenzae*. The predicted relative size and orientation of the *murD* gene is depicted by an arrow in the map.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP344, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP344

SEQ ID NO. 86

pMP344 Length: 2424 nt

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30      1  ACATTAAAAA GGATGAAATT TGGTCAAAGT ATTCGAGAAG AAGGTCCACA
      51  AAGCCATATG AAGAAGACTG GTACACCAAC GATGGGTGGA CTAACATTTC
     101  TATTAAGTAT TGTGATAACG TCTTTGGTGG CTATTATATT TGTAGATCAA
     151  GCWAATCCAA TCATACTGTT ATTATTTGTG ACGATTGGTT TTGGGTAAAT
     201  TGGTTCTTAT ACGATGATTA TATTATTGTT GTTAAAAAGA ATAACCAAGG
35     251  TTTAACAAGT AAACAGAAGT TTTTGGCGCA AATTGGTATT GCGATTATAT
     301  TCTTTGTTTT AAGTAATGTG TTTCAATTGG TGAATTTTTC TACGAGCATA
     351  CATATTCCAT TTACGAATGT AGCAATCCCA CTATCATTTG CATATGTTAT
     401  TTTCAATTGTT TTTTGGCAAG TAGGTTTTTC TAATGCAGTA AATTTAACAG
     451  ATGGTTTAGA TGGATTAGCA ACTGGACTGT CAATTATCGG ATTTACAATG
40     501  TATGCCATCA TGAGCTTTGT GTTAGGAGAA ACGGCAATTG GTATTTTCTG
     551  TATCATTATG TTGTTTGCAC TTTTAGGATT TTTACCATAT AACATTAACC
     601  CTGCTAAAGT GTTTATGGGA GATACAGGTA GCTTAGCTTT AGGTGGTATA
     651  TTTGCTACCA TTTCAATCAT GCTTAATCAG GAATTATCAT TAATTTTTAT
     701  AGGTTTAGTA TTCGTAATTG AAACATTATC TGTTATGTTA CAAGTCGCTA
45     751  GCTTTAAATT GACTGGAAAG CGTATATTTA AAATGAGTCC GATTCATCAT
     801  CATTTTGAAT TGATAGGATG GAGCGAATGG AAAGTAGTTA CAGTATTTTG
  
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      851 GGCTGTTGGT CTGATTTTCAG GTTTAATCGG TTTATGGATT GGAGTTGCAT
      901 TAAGATGCTT AATTATACAG GGTTAGAAAA TAAAAATGTW TTAGTTGTCTG
      951 GTTTGGCAAA AAGTGGTTAT GAAGCAGCTA AATTATTAAG TAAATTAGGT
5    1001 GCGAATGTAA CTGTCAATGA TGGAAAAGAC TTATCACAAG ATGCTCATGC
      1051 AAAAGATTTA GAWTCTATGG GCATTTCTGT TGTAAGTGA AGTCATCCAT
      1101 TAACGTTGCT TGATAATAAT CCAATAATTG TTAAAAATCC TGGAATACCC
      1151 TTATACAGTA TCTATTATTG ATGAAGCAGT GAAACGAGGT TTGAAAATTT
      1201 TAACAGAAGT TGAGTTAAGT TATCTAATCT CTGAAGCACC AATCATAGCT
      1251 GTAACGGGTA CAAATGGTAA AACGACAGTT ACTTCTCTAA TTGGAGATAT
10   1301 GTTTAAAAAA AGTCGCTTAA CTGGAAGATT ATCCGGCAAT ATTGGTTATG
      1351 TTTGCATCTA AAGTWGCACA AGAAGTWAAG CCTACAGATT ATTTAGTTAC
      1401 AGAGTTGTCT TCATTCCAGT TACTTGGAAT CGAAAAGTAT AAACCACACA
      1451 TTGCTATAAT TACTAACATT TATTCGGCGC ATCTAGATTA CCATGRAAAT
      1501 TTAGAAAAC TCAAAAATGC TAAAAAGCAA ATATATAAAA ATCAAACGGA
15   1551 AGAGGATTAT TTGATTTGTA ATTATCATCA AAGACAAGTG ATAGAGTCGG
      1601 AAGAATTAAA AGCTAAGACA TTGTATTTCT CAACTCAAC AAGAAGTTGA
      1651 TGGTATTTAT ATTAAAGATG RTTTTATCGT TTATAAAGGT GTTCGTATTA
      1701 TTAACACTGA AGATCTAGTA TTGCCTGGTG AACATAATTT AGAAAATATA
      1751 TTAGCCAGCT GKGCTKGCTT GTATTTWAGY TGGTGTACCT ATTAAAGCAA
20   1801 TTATTGATAG TTWAAYWACA TTTTCAGGAA TAGAGCATAG ATTGCAATAT
      1851 GTTGGTACTA ATAGAACTTA ATAAATATTA TAATGATTCC AAAGCAACAA
      1901 ACACGCTAGC AACACAGTTT GCCTTAAATT CATTTAATCA ACCAATCATT
      1951 TGGTTATGTG GTGGTTTGGG TCGGAGGGAA TGAATTTGAC GAACTCATTC
      2001 CTTATATGGA AAATGTTTCG CCGATGGTTG TATTCGGACA AACGAAAGCT
25   2051 AAGTTTGCTA AACTAGGTAA TAGTCAAGGG AAATCGGTCA TTGAAGCGAA
      2101 CAATGTCGAA GACGCTGTTG ATAAAGTACA AGATATTATA GAACCAAATG
      2151 ATGTTGTATT ATTGTCACCT GCTTGTGCGA GTTGGGATCA ATATAGTACT
      2201 TTTGAAGAGC GTGGAGAGAA ATTTATTGAA AGATTCCGTG CCCATTTACC
      2251 ATCTTATTAA AGGGTGTGAG TATTGATGGA TGATAAAACG AAGAACGATC
30   2301 AACAAGAATC AAATGAAGAT AAAGATGAAT TAGAATTATT TACGAGGAAT
      2351 ACATCTAAGA AAAGACGGCA AAGAAAAAGW TCCTCTAGAG TCGACCCTGC
      2401 AGGCATGCAA GCTTGGCGTA NCC

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35

Mutant: NT 346

Phenotype: temperature sensitivity

Sequence map: Mutant NT346 is complemented by plasmid
 40 pMP347, which carries a 2.1 kb insert of wild-type *S.*
aureus genomic DNA. A partial restriction map is depicted
 in Fig. 76; no apparent restriction sites for EcoR I, Hind
 III, BamH I or Pst I are present. Database searches at the
 nucleic acid and (putative) polypeptide levels against
 45 currently available databases reveal strong similarities to
 the *tpiS* gene from *B. subtilis*, which encodes triose
 phosphate isomerase (EC 5.3.1.1); similarities are also
 noted to the equivalent gene products from *B. megaterium*

and *B. stearothermophilus*. The predicted relative size and orientation of the *tpiS* gene is depicted by an arrow in the restriction map.

5 **DNA sequence data:** The following DNA sequence data represents the sequence generated by primer walking through clone pMP347, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below
10 can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP347

15

SEQ ID NO. 87

pMP347 Length: 2094 nt

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1  CACATAAACC AGTTGTTGCT ATTTTAGGTG GAGCAAAAGT ATCTGACAAA
20  51  ATTAATGTCA TCAAAAACCT AGTTAACATA GCTGATAAAA TTATCATCGG
    101  CGGAGGTATG GCTTATACTT TCTTAAAAGC GCAAGGTAAA GAAATTGGTA
    151  TTTCATTATT AGAAGAAGAT AAAATCGACT TCGCAAAAGA TTTATTAGAA
    201  AAACATGGTG ATAAAATTGT ATTACCAGTA GACACTAAAG TTGCTAAAGA
    251  ATTTTCTAAT GATGCCAAAA TCACTGTAGT ACCATCTGAT TCAATTCCAG
25  301  CAGACCAAGA AGGTATGGAT ATTGGACCAA ACACTGTAAA ATTATTTGCA
    351  GATGAATTAG AAGGTGCGCA CACTGTTGTT ATGGAATGGA CCTATGGGTT
    401  GTTATTCGAG TTCAGTAACT TTGCACAAGG TACAATTGGT GTTTGTTAAA
    451  GCAATTGCCA ACCTTAAAGA TGCCATTACG ATTATCGGTG GCGGTGATTC
    501  AGCCTGCAGC AGCCATCTCT TTAGGTTTTT GAAAATGACT TCACTCMTAT
30  551  TTCCACTGGT GGCGGCSCKC CATTAGAKTA CCTAGAAGGT WAAGAATGCC
    601  TGGTWTCTMAA GCAAYCAWTA WTAAWTAATA AAGTGATAGT TTAAAGTGAT
    651  GTGGCATGTT TGTTTAACAT TGTACGGGA AAACAGTCAA CAAGATGAAC
    701  ATCGTGTTTC ATCAACTTTT CAAAAATATT TACAAAAACA AGGAGTTGTC
    751  TTTAATGAGA ACACCAATTA TAGCTGGTAA CTGGAAAATG AACAAAACAG
35  801  TACAAGAAGC AAAAGACTTC GTCAATACAT TACCAACACT ACCAGATTCA
    851  AAAGAAKTWR AATCAGTWAT TTGTTGCMCC AGCMATTCAA TTAGATGCAT
    901  TAACTACTGC AGTTWAAGAA GGAAAAGCAC AAGGTTTAGA AATCGGTGCT
    951  CAAAATNCGT ATTTCTGAAGA AATGGGGCTT MACAGTGAAA KTTTCCAGTT
40  1001 GCATAGCAGA TTAGGCTTAA AAAGTTGTAT TCGGTCATTC TGAACCTCGT
    1051 GAATATTCCA CGGAACCAGA TGAAGAAATT AACAAAAAAG CGCACGTATT
    1101 TTCAAACATG GAATGAMTCC AATTATATGT GTTGGTGAAA CAGACGAAGA
    1151 GCGTGAAAGT GGTAAAGCTA ACGATGTTGT AGGTGAGCAA GTTAAAGAAA
    1201 GCTGTTGCAG GTTTATCTGA AGATCAAAC TAAATCAGTT GTAATTGCTT
    1251 ATGAACCAAT CTGGGCAATC GGAACCTGGTA AATCATCAAC ATCTGAAGAT
45  1301 GCAAATGAAA TGTGTGCATT TGTACGTCAA ACTATTGCTG ACTTATCAAG
    1351 CAAAGAAGTA TCAGAAGCAA CTCGTATTCA ATATGGTGGT AGTGTTAAAC
    1401 CTAACAACAT TAAAGAATAC ATGGCACAAA CTGATATTGA TGGGGCATT
    1451 GTAGGTGGCG CATCACTTAA AGTTGAAGAT TTCGTACAA TGTTAGAAGG

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1501 TGCAAAATAA TCATGGCTAA GAAACCAACT GCGTTAATTA TTTTAGATGG
1551 TTTTGCGAAC CGCGAAAGCG AACATGGTAA TGCGGTAAAA TTAGCAAACA
1601 AGCCTAATTT TTNGATCGGT TNATTACCAA CCAAATATCC CAACCGAACT
1651 TCAAAATTCG AAGGCGAGTG GCTTAAGATG TTGGACTACC CTGAAGGACA
5 1701 AATGGGTAAC TCAGAAGTTG GTCATATGAA TATCGGTGCA GGACGTATCG
1751 TTTATCAAAG TTTAACTCGA ATCAATAAAT CAATTGAAGA CCGTGATTTT
1801 TTTGAAAATG ATGTTTTTAA TAATGCAATT GCACACGTGA ATTCACATGA
1851 TTCAGCGTTA CACATCTTTG GTTTATTGTC TGACGGTGGT GTACACAGTC
1901 ATTACAAACA TTTATTTGCT TTGTTAGAAC TTGCTAAAAA ACAAGGTGTT
10 1951 GAAAAAGTTT ACGTACACGC ATTTTATAGAT GGCCGTGACG TAGATCAAAA
2001 ATCCGCTTTG AAATACATCG AAGAGACTGA AGCTAAATTC AATGAATTAG
2051 GCATTGGTCA ATTTGCATCT GTGTCTGGTC GTTATTATGC ANTG

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15

Mutant: NT348**phenotype:** temperature sensitivity

Sequence map: : Mutant NT348 is complemented by plasmid pMP649, which carries a 3.3 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 77; no apparent restriction sites for EcoR I, Hind III, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal DNA sequence identities to two different Genbank entries for *S. aureus* DNA. The left-most contig below matches Genbank Accession No. U31979, which includes the complete *aroC* gene, encoding 5-enolpyruvylshikimate 3-phosphate phospholyase (EC 4.6.1.4), and the N-terminal portion of the *aroB* gene, encoding 5-dehydroquinate hydrolyase (EC 4.2.1.10); the right-most contig matches Genbank Accession No. L05004, which includes the C-terminal portion of the *aroB* gene. Neither Genbank entry described contains the complete DNA sequence of pMP649. Further experiments are underway to determine whether one or both of the genes identified in clone pMP649 are essential.

DNA sequence data: The following DNA sequence data represents the sequence generated from clone pMP649, starting with standard M13 forward and M13 reverse sequencing primers; the sequence contig will be completed via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP649

SEQ ID NO. 88

5 pMP649.forward Length: 954 nt

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      1 GGGGWYYCTC TAGAGYCGAC CTRCAGGCAT SCAAGCTTBA CCAGGWTCAA
    51 TTAGAGGTRA TTWAGGTTTA RCTKTTSQTV GAADTATCAT BMTCCGGTTCA
  101 GATTCCTGAG AGTCTGCTGA ACGTGAAATT AATCTATGGT TTAATGAAAA
  151 TGAAATTACT AGCTATGCTT CACCACGTGA TGCATGGTTA TATGAATAAA
  201 ATATAAACTG TAAACCTTTA CGATTTATTT ATAAAGGTAG AAAGGGTTTT
  251 GTTATGTGGT TAGTCATTAT GATTATACAT AACCAAGGCC GTTTTTTATG
  301 TTGTAGTAAA TTACTTGAAA AATTTTATAG TTTTGTGGTA ACACGTATTA
  351 AAAAGAGAGG AATATTCTTT ATCAAAATGAA ACTAAACAGA GAGAAGGGGT
  401 TGTTAAAATG AAGAATATTA TTTCGATTAT TTTGGGGATT TTAATGTTCT
  451 TAAAATTAAT GGAATTACTA TATGGTGCTA TATTTTTFAGA TAAACCACTT
  501 AATCCTATAA CAAAATTAT TTTTATACTG ACTCTCATTT ATATTTTTTA
  551 TGTATTAGTA AAAGAATTGA TTATATTTTT GAAGTCAAAG TATAACAAAA
  601 GCGCTTAACA TATGTTTATT TTAATATCAT AATTTTTTTA AACGGGACTG
  651 ATTAACYTTT ATTAATAATT AACAGTTCGT TCTTTTGTAT TAAGAAATGT
  701 AGTCAGTATA TTATTTGCTA AAGTTGCGAT ACGATTATAT TAAAACGGCT
  751 AATCATTTTT AATTAATGAT TATATGATGC AACTGTTTAG AAATTCATGA
  801 TACTTTTCTA CAGACGAATA TATTATAATT AATTTTAGTT CGTTTAATAT
  851 TAAGATAATT CTGACATTTA AAATGAGATG TCATCCATTT TCTTAATTGA
  901 GCTTGAAAAC AAACATTTAT GAATGCACAA TGAATATGAT AAGATTAACA
  951 ACAT

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SEQ ID NO. 89

pMP649.reverse Length: 841 nt

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  30 1 CTTTMAWKRC CTRAACCACT TAACAAACCT GCCAATAATC GTGTTGTCGT
    51 ACCAGAATTA CCTGTATACA ATACTTGATG TGGCGTGTTA AAAGATTGAT
  101 ATCCTGGGGA AGTCACAACT AATTTTTTCAT CATCTTCTTT GATTTCTACA
  151 CCTAACAGTC GGAAAATGTC CATCGTACGA CGACAATCTT CGCCAAGTAG
  35 201 TGGCTTATAT ATAGTAGATA CACCTTCAGC TAGCGACGCC AACATGATTG
    251 CACGGTGTGT CATTGACTTA TCGCCCGGCA CTTCTATTTT GCCCTTTAAC
    301 GGACCTGAAA TATCAATGAT TTGTTTCATTT ACCATTTTCAT TCACCTACTT
    351 AAAATATGTT TTTAATTGTT CACATGCATG TTGTAATGTT AGTTGATCAA
    401 CATGTTGTAC AACGATATCT CCAAATTGTC TAATCAAGAC CATTTGTACA
  40 451 CCTTGCTTAT CATTCTTTTT ATCACTTAGC ATATATTGGT ATAACGTTTC
    501 AAAATCCAAG TCAGTTATCA TGTCTAAAGG ATAGCCGAGT TGTATTAAAT
    551 ATTGAATATA ATGATTAATA TCATGCTTAG RATCAAACAA AGCATTCGCA
    601 ACTATAAATT GATAGATAAT GCCAACCATC ACTGACATGA CCATGAGGTA
    651 TTTTATGATA GTATTCAACA GCATGACCAA ATGTATGACC TAAATTTAAR
  45 701 AATTTACGTA CACCTTGTTT TTTTTSATCT GGCGAATAAC AATATCCAGC
    751 TTSGTTTCAA TACCTTTRGS AATWTATTTR TCCATACCAT TTAATGACTG
    801 TAATATCTCT CTATCTTTAA AGTGCTGTTC GATATCTTGC G

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50

Mutant: NT359

phenotype: temperature sensitivity

Sequence map: : Mutant NT359 is complemented by plasmid
 5 pMP456, which carries a 3.2 kb insert of wild-type *S.*
aureus genomic DNA. A partial restriction map is depicted
 in Fig. 78; no apparent restriction sites for EcoR I, Hind
 III, BamH I or Pst I are present. Database searches at the
 nucleic acid and (putative) polypeptide levels against
 10 currently available databases reveal identity to the *glnRA*
 locus of *S. aureus* (Genbank Accession No. X76490), also
 referred to as the *femC* locus; mutations localized to *femC*
 have been reported in the scientific literature to display
 an increased sensitivity to the bacterial cell-wall
 15 synthesis inhibitor methicillin.

DNA sequence data: The following DNA sequence data
 represents the sequence generated from clone pMP456,
 starting with standard M13 forward and M13 reverse
 20 sequencing primers; the sequence contig will be completed
 via primer walking strategies. The sequence below can be
 used to design PCR primers for the purpose of amplification
 from genomic DNA with subsequent DNA sequencing.

25 clone pMP456

SEQ ID NO. 90

pMP456.forward Length: 568 nt

```

30      1 CCGGGGATCC TCTAGAGTCG ATCTTTGCAT TCTTTAAGCT TAAATTTTCT
      51 ATTCTTCTTT CTCTACGGCG CATAGCATTA ATATTACCGT AACTTATCCC
     101 AGTATCTTTA TTAATTTGAT AACTCGATAT CTCTTTGTTT TCTATCAATT
     151 CTTTGATTGT ATTGAATATT TCATCATAGC AATTCATAAA TTAGATGAGG
     201 CGAAATTTTT AATTTTTTTAG AATATCAATA GTANTATAAC TAAATGAAA
35     251 ATACCGATCG ATAAACAAAA AGATATTTTT TGTTTTGTTT CTCTTTTCAT
     301 ATAGTATTAC CCCCTTAATA ATGCGTAGTA AGGTCCCTCT TTTCGGGGTC
     351 TTACCTTANA AACGTTCTGC AAATGAATTC GATGAGAAGT AATATGAATA
     401 TGGCTATTTT CAAGTAATAC TCAACGTTTT CGCGACGTTT TTTTATCGCC
     451 TCATCTCATC ACCTCCAAAT ATATTAAAAT TCATGTGAAC TAAATATATA
40     501 AATGGTCTTC CCCAGCTTTA AAAAAATAAA TACATAAAAC ATTTTACTTG
     551 GACCAAAACT TGGACCCC
  
```

SEQ ID NO. 91

pMP456.reverse Length: 581 nt

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45      1 ATGCCTGCAG GTCGATCATT AATTAAAAAC CCTGGCGGTG GTTTAGCTAA
  
```

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51  GATTGGTGGG TACATTGCTG GTAGAAAAGA TTTAATTGAA CGATGTGGTT
101 ATAGATTGAC AGCACCTGGT ATTGGTAAAG AAGCGGGTGC ATCATTAAT
151 GCATTGCTTG AAATGTATCA AGGTTTCTTT TTAGCACCAC ACGTTGTCAG
201 TCAGAGTCTT AAAGGTGCAT TGTTTACTAG TTTATTTTGA GAAAAAATGA
5  251 ATATGAACAC AACGCCGAAG TACTACGAAA AACGAACTGA TTTAATTCAA
301 ACAGTTAAAT TTGAAACGAA AGAACAAATG ATTTCAATTT GTCAAAGTAT
351 TCAACACGCA TCCCAATTA ATGCACATTT TAGTCCANAA CCTAGTTATA
401 TGCCTGGTTA CGAAGATGAT GTTATTATGG CAGCTGGTAC GTTTATTCAA
451 GGTTTCATCCG ATTGAATTAT CTGCAGATGG ACCTATTCGT CCTCCTTATG
10 501 AAGCATATGT TCAAGGANGA TTAACATATG AACACGTTAA AATTGCTGTT
551 GACAAGANCT GTTTAATCAG TTTGAAAAAA C

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15

Mutant: NT371**phenotype:** temperature sensitivity**Sequence map:** : Mutant NT371 is complemented by plasmid pMP461, which carries a 2.0 kb insert of wild-type *S.*

20 *aureus* genomic DNA. A partial restriction map is depicted in Fig. 79. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level similarities to *yluD*, a hypothetical ABC transporter (Genbank Accession No. M90761), and *yidA*, a hypothetical ORF of unknown function (Genbank Accession No. L10328).

30 **DNA sequence data:** The following DNA sequence data represents the sequence generated by primer walking through clone pMP461, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA

35 sequencing.

clone pMP461

SEQ ID NO. 92

40 pMP461 Length: 2001 nt

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1  CGGGGATCCT CTAAAGTCGA TCAAATTGGG CGAATGAAGC AAGGAAAAAC
51  AATTTTAAAA AAGATTTCTT GGCAAATTGC TAAAGGTGAT AAATGGATAT
101 TATATGGGTT GAATGGTGCT GGCAAGACAA CACTTCTAAA TATTTTAAAT
45 151 GCGTATGAGC CTGCAACATC TGGAAGTGT AACCTTTTCG GTAAATGCC
201 AGGCAAGGTA GGGTATTCTG CAGAGACTGT ACGACAACAT ATAGGTTTTG

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5 251 TATCTCATAG TTTACTGGAA AAGTTTCAAG AGGGTGAAAG AGTAATCGAT
 301 GTGGTGATAA GCGGTGCCCTT TAAATCAATT GGTGTTTATC AAGATATTGA
 351 TGATGAGATA CGTAATGAAG CACATCAATT ACTTAAATTA GTTGAATGT
 401 CTGCTAAAGC GCAACAATAT ATTGGTTATT TATCTACCGG TGAAAAACAA
 451 CGAGTGATGA TTGCACGAGC TTTAATGGGG CAACCCAGG TTTTAATTTT
 501 AGATGAGCCA GCAGCTGGTT TAGACTTTAT TGCACGAGAA TCGTTGTTAA
 551 GTATAC TTGA CTCATTGTCA GATTCATATC CAACGCTTGC GATGATTAT
 601 GTGACGCACT TTATTGAAGA AATAACTGCT AACTTTTCCA AAATTTTACT
 651 GCTAAAAGAT GGCCAAAGTA TTCAACAAGG CGCTGTAGAA GACATATTAA
 10 701 CTTCTGAAAA CATGTCACGA TTTTCCAGA AAAATGTAGC AGTTCAAAGA
 751 TGGAATAATC GATTTTCTAT GGCAATGTTA GAGTAAATAT TTTGCAAATA
 801 ATAAGTAATA ATGACAAAAT TTAATTAAGA TAAATGGAC AGTGGAGGGC
 851 AATATGGATA ACGTTAAAAG CAATATTTTT GGACATGGAT GGAACAATTT
 901 TACATTGAAA ATAATCCAAG CATCCAACGT WTACGAAAGA TGTTCAATTAA
 15 951 TCAATTGGAG AGAGAAAGGA TATWAAGTAT TTTTGGSCAA CAGGACGTTT
 1001 GCATTCTGAA ATACATCMAA YTTGTACCTC AAGATTTTGC GGTTAATGGC
 1051 ATCATTAGTT CAAATGGAAC AATTGGAGAA GTAGATGGAG AAATTATCTT
 1101 CAAGCATGGT TTATCATTGG CTCAAGTGCA ACAAATTACT AATTAGCTA
 1151 AGCGCCAACA AATTTATTAT GAGGTATTTT CTTTGAAGG TAATAGAGTT
 20 1201 TCTTTAAAAG AAGATGAAAC ATGGATGCGA GATATGATTC GTAGTCAAGA
 1251 TCCTATTAAT GCGGTAAGTC ATAGTGAATG GTCTTCAAGA CAAGATGCGC
 1301 TTGCTGGTAA GATAGATTGG GTAAC TAAGT TCCCTGAAGG TGAATATTCA
 1351 AAAATTTATC TATTCAGTTC TAATTTAGAA AAAATAACAG CATTTAGAGA
 1401 TGAATTAAAG CAAAATCATG TGCAACTACA GATTAGTGTT TCAAATTCAT
 25 1451 CAAGATTTAA TGCGGAAACA ATGGCTTATC AAAGTATAA AGGTACAGGC
 1501 ATTAAGAAA TGATTGCACA TTTTGGTATT CATCAAGAAG AAACGTTAGT
 1551 TATTGGAGAT AGCGACAATG ATAGAGCAAT GTTTGAATTT GGTCATTATA
 1601 CAGTTGCTAT GAAAAATGCA CGCCCTGAAA TCCAAGCATT AACTTCAGAT
 1651 GTAACGGCAT ACACGAATGA AGAGGATGGC GCAGCAAAAT ATTTAGCAGA
 30 1701 GCATTTTTTA GCTGAATAAT AAAATAGGTA GTTATTTATT ATTTAATTTA
 1751 CAATAGTTGA TGAGTAATGT ACAAAGAGCA GTAAAGTTAT TTTCTATTAG
 1801 AAAATGTCTT ACTGCTCTTT TGTATGCTTA TAAATATTTG AATCATCTAT
 1851 ATTTAATTGG ACAAACCTTA TGAGAATAAA TATTGTTAAA ACTAATAAGA
 1901 TAGGAAATTC ATTGATTTTG AATAATATTT CTTGTTTTAA GGTTTAACTA
 35 1951 TTGAATTGTA TACTTCTTTT TTTAGTAGCA ACAGATCGAC CTGCAGGCAT
 2001 A

40

Mutant: NT 379

Phenotype: temperature sensitivity

Sequence map: Mutant NT379 is complemented by plasmid pMP389, which carries a 2.5 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 80; no apparent restriction sites for EcoR I, Hind III, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong similarities to

45

the tagF gene from *B. subtilis*, which encodes a protein involved in the biosynthesis of teichoic acid polymers (Genbank Accession No. X15200). The Tag genes of *B. subtilis* have been identified as essential and are expected to make good candidates for screen development. The predicted relative size and orientation of the tagF gene is depicted by an arrow in the restriction map.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP389, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP389

20 SEQ ID NO. 93

pMP389 Length: 2522 nt

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      1  GANCTCGGTA CCCGGGGATG CCTSYAGAGT CGATCGCTAC CACCTTGAAT
      51  GACTTCAATT CTTTCATCAG AAATTTTGAA TTTTCTAAGT GTATCTTTTCG
25     101  TATGCGTCAT CCATTGTTGT GGCCTCGCGA TAATAATTTT TTCAAATCA
      151  TTAATTAAAA TAAATTTTTC TAATGTATGG ATTAAATCG GTTTGTTGTC
      201  TAAATCTAAA AATTGTTTAG GTAAAGGTAC GTTACCCATT CTTGAGCCTA
      251  TACCTCCAGC TAGAATACCA GCGTATTTCA TAAAATACTT CCTCCATTCA
      301  ACTATATCTA TATTTAATTA TTTAAATTTT GTTGCATTTT CCAATTGAAA
30     351  ACTCATTTTA AAATCAAAC TCTAAATGTC TGTGTATTAC TTAAATTAT
      401  ACATATTTTG CTTATATTTT AGCATATTTT GTTTAAACCT ATATTACATT
      451  ATATCAGACG TTTTCATACA CAAATAATAA CATACAAGCA AACATTTTCGT
      501  TTATTATTTA TATCACTTAA CTAATTAATT TATAATTTT TATTGTTTTT
      551  AAGTTATCAC TTAAAAATCG TTTGGCAAAT TCGTTGTGAC GCTTGTCCAT
35     601  CTTCTAATGA ACAGAATTTT TGATAAAATA CCGTTCGTGC TTCAATATAC
      651  TCATTTGCAG TCTCATCGAT TTGTTTAAAT GCATCAATGA GTGCTGTTTG
      701  ATTTTCAACA ATTGGAMCTG GCAACTCTTT TTTATAATCC ATGTAAAAAC
      751  CTCTAAGCTC ATCGCCATAT TTATCTAAGT CATATGCATA GAAAATTTGC
      801  GGACGCTTTA ATACACCGAA GTCGAACATG ACAGATGAGT AGTCGGTAAC
40     851  TAACGCATCG CTGATTAAGT TATAAATCCG AAATGCCTTC ATAATCTGGA
      901  AAMGTCTTTC AACAAAATCA TCAATGTTCA TCAATAACGY GTCAACAAC
      951  AAATAATGCA KCGGTAATAA AATAACATAA TCATCATCCA GCGCTTGACG
     1001  CAAAGCTTCT ATATCAAAGT TAACATTAAA TTGATATGAA CCCTTCTCGG
     1051  AATCGCTTCA TCGTCAACGC CAAGTTGGCG CGTACATAAT CAACTTTTTT
45     1101  ATCTAATGGA ATATTTAATC TTGTCTTAAT ACCATTAATA TATTCAGTAT
     1151  CATTGCGTTT ATGTGATAAT TTATCATTTT TTGGATAACC TGTTTCCAAA
     1201  ATCTTATCTC GACTAACATG AAATGCATTT TGAAATATCG ATGTCGAATA

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1251 TGGATTAGGT GACACTAGAT AATCCCACCG TTGGCTTTCT TTTTAAAGC
1301 CATCTTGGTA ATTTTGAGTA TTTGTTCTTA GCATTTTAAC GTTACTAATA
1351 TCCAAACCAA TCTTTTTTAA TGGCGTGCCA TGCCATGTTT GTAAGTACGT
1401 CGTTTCGGGT GATTTATATA ACCAATCTGG TGTACGTGTG TTAATCATCC
5. 1451 ACGCTTTCGC TCTTGGCATC GCTAAAAACC ATTTTCATTGA AAACTTTGTA
1501 ACATATGGTA CATTGTGCTG TTGGAATATG TGTTTCATATC CTTTTTTCAC
1551 ACCCCATATT AATTGGGCAT CGCTATGTTT AGTTAAGTAT TCATATAATG
1601 CTTTGGGGTT GTCGCTGTAT TGTTTACCAT GAAAGCTTTC AAAATAAATT
1651 AGATTCTTGT TTGGCAATTT TGGATAGTAA TTTAAAAGTC GTATATATAC
10 1701 TATGTTCTAT CAATTTTTTA ATTGTATTTT TAATCATGTC GTACCTCCGA
1751 CGTGTTTTTG TAATTATATT AATATGTATG AGCAAGCTCA TTGTAACCAT
1801 GCCTATTATA GCATTTTCATC ATAAAATACA TTTAACCATT ACACCTGTCG
1851 TTAATTATCA TACGAAATAC ATGATTAATG TACCACTTTA ACATAACAAA
1901 AAATCGTTAT CCATTCATAA CGTATGTGTT TACACATTTA TGAATTAGAT
15 1951 AACGATTGGA TCGATTATTT TATTTWACAA AATGACAATT CAGTTGGAAG
2001 GTGATTGCTT TTGATTGAAT CGCCTTATGC ATGAAAAATC AAAAGGTTAT
2051 TCTCATTGTA TAGTCCTGCT TCTCATCATG ACATGTTGCT CACTTCATTG
2101 TCAGAACCCT TCTTGAAAAC TATGCCCTAT GACTCATTTG CATGGCAAGT
2151 AATATATGCC AACATTAGCG TCTAAACAAA TCTTTGACTA AACGTTCACT
20 2201 TGAGCGACCA TCTTGATATT TAAAATGTTT ATCTAAGAAT GGCACAACCT
2251 TTTCAACCTC ATAATCTTCA TTGTCCAAAG CATCCATTAA TGCATCAAAG
2301 GACTGTACAA TTTTACCTGG AACAAATGAT TCAAATGGTT CATAGAAATC
2351 ACGCGTCGTA ATGTAATCTT CTAAGTCAAA TGCATAGAAA ATCATCGGCT
2401 TTTTAAATAC TGCATATTCA TATATTAAAG ATGAATAATC ACTAATCAAC
25 2451 AAGTCTGTAA CAAAGAGAAT ATCGTTWACT TCASGRTCGA TCGACTCTAG
2501 AGGATCCCCG GGTACCGAGC TC

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30

Mutant: NT 380

Phenotype: temperature sensitivity

Sequence map: Mutant NT380 is complemented by plasmid pMP394, which carries a 1.3 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 81. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong similarities to the *cdsA* gene product from *E. coli* (Genbank Accession No. M11330), which encodes phosphatidate cytidyltransferase (EC 2.7.7.41); the *cdsA* gene product is involved in membrane biogenesis and is likely to be a good candidate for screen development. The predicted relative size and orientation of the *cdsA* gene is depicted by an arrow in the restriction map.

45

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP394, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

10 **clone pMP394**

SEQ ID NO. 94

pMP394 Length: 1335 nt

```

15      1  CAGAGTTGTT AATTCGTACT TCAGGAGAAC AAAGAATAAG TAATTTCTTG
      51  ATTTGGCAAG TTTCGTATAG TGAATTTATC TTTAATCAAA AATTATGGCC
     101  TGACTTTGAC GAAGATGAAT TAATTAAATG TATAAAAATT TATCAGTCAC
     151  GTCAAAGACG CTTTGGCGGA TTGARTGAKG AGKATRTATA GTATGAAAGT
     201  TAGAACGCTG ACAGCTATTA TTGCCTTAAT CGTATTCTTG CCTATCTTGT
20      251  TAAAAGGCGG CCTTGTGTGA ATGATATTG CTAATATATT AGCATTGATT
     301  GCATTAAAAG AAATTGTTGA ATATGAATAT GATTAAATTT GTTTCAGTTC
     351  CTGGTTTAAT TAGTGCAGTT GGTCTTATCA TCATTATGTT GCCACAACAT
     401  GCAGGGCCAT GGGTACAAGT AATTCAATTA AAAAGTTTAA TTGCAATGAG
     451  CTTTATTGTA TTAAGTTATA CTGTCTTATC TAAAAACAGA TTTAGTTTTA
25      501  TGGATGCTGC ATTTTGCTTA ATGTCTGTGG CTTATGTAGG CATTGGTTTT
     551  ATGTTCTTTT ATGAAACGAG ATCAGAAGGA TTACATTACA TATTATATGC
     601  CTTTTTAATT GTTTGGCTTA CAGATACAGG GGCTTACTTG TTTGGTAAAA
     651  TGATGGGTTA AACATAAGCT TTGGCCAGTA ATAAKTCCGA ATAAAACAAT
     701  CCGAAGGATY CATAGGTGGC TTGTTCTGTA GTTGATAGT ACCACTTGCA
30      751  ATGTTATATT TTGTAGATTT CAATATGAAT GTATGGATAT TACTTGGAGT
     801  GACATTGATT TTAAGTTTAT TTGGTCAATT AGGTGATTTA GTGGAATCAG
     851  GATTTAAGCG TCATTTNGGC GTTAAAGACT CAGGTCGAAT ACTACCTGGA
     901  CACGGTGGTA TTTTAGACCG ATTTGACAGC TTTATGTTTG TGTTACCATT
     951  ATTAAATATT TTATTAATAC AATCTTAATG CTGAGAACAA ATCAATAAAC
35     1001  GTAAAGAGGA GTTGCTGAGA TAATTTAATG AATCCTCAGA ACTCCCTTTT
     1051  GAAAATTATA CGCAATATTA ACTTTGAAAA TTATACGCAA TATTAACTTT
     1101  GAAAATTAGA CGTTATATTT TGTGATTTGT CAGTATCATA TTATAATGAC
     1151  TTATGTTACG TATACAGCAA TCATTTTTAA AATAAAAAGAA ATTTATAAAC
     1201  AATCGAGGTG TAGCGAGTGA GCTATTTAGT TACAATAATT GCATTTATTA
40     1251  TTGTTTTTGG TGTACTAGTA ACTGTTTCATG AATATGGCCA TATGTTTTTT
     1301  GCGAAAAGAG CAGGCATTAT GTGTCCAGAA TTTGC

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45

Mutant: NT401

phenotype: temperature sensitivity

- Sequence map:** Mutant NT401 is complemented by plasmid pMP476, which carries a 2.9 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 82. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal sequence identity in the middle of the clone to pMP64, the complementing clone to NT31 (described previously). Since pMP64 does not cross complement NT401, and pMP476 contains additional DNA both upstream and downstream, the essential gene is likely to reside in the flanking DNA. The remaining DNA that completely contains an ORF is that coding for *yqeJ*, a hypothetical ORF from *B. subtilis* (Genbank Accession No. D84432)
- DNA sequence data:** The following DNA sequence data represents the sequence generated by primer walking through clone pMP476, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP476

25

SEQ ID NO. 95

pMP476 Length: 2902 nt

```

      1  GAGCTCGGTA CCCGGGGATC CTCTAGAGTC GATCATTACC TAATTCGTAT
30      51  TGTCGAACAA TTTGATACAT TTTACCTAAA TCATCATATT TACAGAAATC
      101  ATGTAATACA CCTGCTAATT CTACTTTACT AGTGTCTCCA TCATAAATTT
      151  CTGCCRATTT AATCGCTGTT TCTGCAACTC TTAAAGAATG ATTGATRACG
      201  TTTCTCTGGA CAGTTTCTCT TTTGCAAGCC GTTTTGCTTT TTCAATGTWC
      251  ATATAATCCT TCCCCCTTAA TATAGTTTTT AACGGATTTA GGAACAAGAA
35      301  CTTGGATAGA TTTCCCTTCA CTAACCTCTT GTCGAATCAT TGTCGAACTT
      351  ATATCTACCC TAGGTATCTG AATTGCAATC ATAGCATTTT CAACATTTTG
      401  ACTATTTTGT TCTCGATTTA CAACTACAAA AGTAACCATT TCTTTTAAGT
      451  ATTCAATTTG ATACCATTTC TCTAGTTGGT TATACTGATC CGTCCCAATA
      501  ACAAAGTACA ACTCACTGTC TTTGTGTTGC TCCTTGAATG CCTTGATCGT
40      551  GTCATAGGTA TAACTTTGAC CACCACGTTT AATTTCATCG TCACAAATAT
      601  CTCCAAAACC AAGCTCGTCG ATAATCATCT GTATCATTTG TAATCTGTGC
      651  TGAACGTCTA TAAAATCATG GTGCTTTTTT AATGGAGAMA WAAAAMWARR
      701  WAAAAAATAA AATTCATCTG GCTGTAATTC ATGAAATACT TCGCTAGCTA
      751  CTATCATATG TTGCAGTATG GATAGGGTTA AACTGACCGC CGTAAAGTAC
45      801  TATCTTTTTC ATTATTATGG CAATTCAATT TCTTTATTAT CTTTAGATTC
      851  TCTATAAATC ACTATCATAG ATCCAATCAC TTGCACTAAT TCACTATGAA

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5  901  KTAGCTTCCG  CTTAATGTTT  CCAGCTAATY  CTTTTTTATC  ATCAAAGTTT
    951  ATTTTGTTAK  TACATGTTAC  TTTAATCAAT  YCTCTGTTTT  CYAACGTTAT
1001  CATCTATTTG  TTTAATCATA  TTTTCGTTGA  TACCGCCTTT  TCCAATTTGA
1051  AAAATCGGAT  CAATATTGTG  TGCTAAACTT  CTTAAGTATC  TTTTTGTGTT
1101  GCCAGTAAGC  ATATGTTATT  CTCCTTTTAA  TTGTTGTAAA  ACTGCTGTTT
1151  TCATAGAATT  AATATCAGCA  TCTTTATTAG  TCCAAATTTT  AAAGCTTTCC
1201  GCACCCTGGT  AAACAAACAT  ATCTAAGCCA  TTATAAATAT  GGTTTCCCTT
1251  GCGCTCTGCT  TCCTCTAAAA  TAGGTGTTTT  ATACGGTATA  TAAACAATAT
1301  CACTCATTAA  AGTATTGGGA  GAAAGAGCTT  TAAATTAATA  ATACTTTCGT
10  1351  TATTTCCAGC  CATACCCGCT  GGTGTTGTAT  TAATAACGAT  ATCGAATTCA
1401  GCTAAATACT  TTTTCAGCAT  TGCTAATGAA  ATTTGGTTTA  TATTTAAATT
1451  CCAAGATTCA  AAACGAGCCA  TCGTCTTATT  CGCAACAGTT  AATTTGGGCT
1501  TTACAAATTT  TGCTAATTCA  TAAGCAATAC  CTTTACTTGC  ACCACCTGCG
1551  CCCAAAATTA  AAATGTATGC  ATTTTCTAAA  TCTGGATAAA  CGCTGTGCAA
15  1601  TCCTTTAACA  TAACCAATAC  CATCTGTATT  ATACCCTATC  CACTTGCCAT
1651  CTTTATCAA  AACAGTGTTA  ACTGCACCTG  CATTAATCGC  TTGTTTCATCA
1701  ACATAATCTA  AATACGGTAT  GATACGTTCT  TTATGAGGAA  TTGTGATATT
1751  AAASCCTTCT  AATTYTTTTT  TSGAAATAAT  TTCTTTAATT  AAATGAAAAA
1801  TTYTTCAATT  GGAATATTTT  AAAGCTTCAT  AAGTATCATC  TTAATCCTAA
20  1851  AGAATTAATA  TTTGCTCTAT  GCATAACGGG  CGACAAGGAA  TGTGAAATAG
1901  GATTTCTTAT  AACTGCAAAT  TTCATTTTTT  TAATCACCTT  ATAAAATAGA
1951  ATTYTTTAAT  ACAACATCAA  CATTTTtagg  AACACGAACG  ATTACTTTAG
2001  CCCCTGGTCC  TATAGTTATA  AAGCCTAGAC  CAGAGATCAT  AACATCGCGT
2051  TTCTCTTTGC  CTGTTTCAAG  TCTAACAGCC  TTTACCTCAT  TAAGATCAAA
25  2101  ATTTTGTGGA  TTTCCAGGTG  GCGTTAATAA  ATCGCCAAGT  TGATTACGCC
2151  ATAAATCATT  AGCCTTCTCC  GTTTTAGTAC  GATGTATATT  CAAGTCATTA
2201  GAAAAGAAAC  AAACAAACG  ACGTTTACCA  CCTGAWACAT  AATCTATGCG
2251  CGCTAGACCG  CCGAAGAATA  ATGTCKGCGC  CTCATTTAAT  TGATATACGC
2301  GTTGTTTTAT  TTCTTTCTTA  GGCATAATAA  TTTTCAATYC  TTTTTCATA
30  2351  ACTAAATGCG  TCATTGGGTG  ATCTTGAATA  ATACCTGGTG  TATCATACAT
2401  AAATGATGTT  TCATCTAAAG  GAATATCTAT  CATATCTAAA  GTTGYTTCCA
2451  GGAATCTTG  AAGTTGTTAC  TACATCTTTT  TCACCAACAC  TAGCTTCAAT
2501  CAGTTTATTA  ATCAATGTAG  ATTTCCCAAC  ATTCGTTGTC  CCTACAATAT
2551  ACACATCTTC  ATTTTCTCGA  ATATTCGCAA  TTGATGATAA  TAAGTCGTCT
35  2601  ATGCCCCAGC  CTTTTTCAGC  TGAATTAAT  ACGACATCGT  CAGCTTCCAA
2651  ACCATATTTT  CTTGCTGTTC  GTTTTAACCA  TTCTTTAACT  CGACGTTTAT
2701  TAATTTGTTT  CGGCAATAAA  TCCAATTTAT  TTGCTGCTAA  AATGATTTTT
2751  TTGTTTCCGA  CAATACGTTT  AACTGCATTA  ATAAATGATC  CTTCAAAGTC
2801  AAATACATCC  ACGACATTGA  CGACAATACC  CTTTTTATCC  GCAAGTCCTG
40  2851  ATAATAATTT  TAAAAAGTCT  TCACTTTCTA  ATCCTACATC  TTGAAGTTTCG
    2901  TT

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45

Mutant: NT423

phenotype: temperature sensitivity

Sequence map: : Mutant NT423 is complemented by plasmid pMP499, which carries a 2.0 kb insert of wild-type *S.*

50 *aureus* genomic DNA. A partial restriction map is depicted

in Fig. 83. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level similarities to *yqhY*, a hypothetical ORF identified from a genomic sequencing effort in *B. subtilis* (Genbank Accession No. D84432), and *yqhZ*, a hypothetical ORF from *B. subtilis* bearing similarity to the *nusB* gene product from *E. coli* (Genbank Accession No. M26839; published in Imamoto, F. et al. *Adv. Biophys.* 21 (1986) 175-192). Since the *nusB* gene product has been demonstrated to be involved in the regulation of transcription termination in *E. coli*, it is likely that either one or both of the putative genes identified in this sequence contig encode essential functions.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP499, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP499

SEQ ID NO. 96

pMP499 Length: 1916 nt

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1  AGTCGATCAA AGCCAATGTT CCAGTTGTTC CTGGTAGTGA CGGTTTAATG
30  51  AAAGACGTCT CAGAAGCTAA GAAAATCGCC AAAAAAATTG GCTATCCGGT
    101  CATCATTA AAA GCTACTGCTG GCGGTGGCGG AAAAGGTATC CGTGTGCTC
    151  GTGATGAAAA AGAACTTGAA ACTGGCTTCC GAATGACAGA ACAAGAAGCT
    201  CAAACTGCAT TTGGTAATGG TGGACTTTAT ATGGAGAAAT TCATCGAAAA
    251  CTTCCGCCAT ATTGAAATCC AAATTGTTGG GGACAGCTAT GGTAAATGTAA
35  301  TTCATTTAGG AGAACGTGAT TGTACAATTC AAAGACGTNT GCAGAAATTA
    351  GTGGAAGAAG CACCTTCCCC NATTTTAGAT GATGAAACAC GTCGTGAAAT
    401  GGGAAATGCC GCAGTTCGTG CAGCGAAAGC TGTAATTTAT GAAAATGCCG
    451  GAACAATTGA GTTTATATAT GATTAAATG ATAATAAATT TTATTTTATG
    501  GAAATGAATA CACGTATTCA AGTAGAACAT CCTGTAAC TG AATGGTAAC
40  551  AGGAATTGAT TTAGTTAAAT TACAATTACA AGTTGCTATG GGTGACGTGT
    601  TACCGTATAA ACAAGAAGAT ATTAAATTAA CAGGACACGC AATTGAATTT
    651  AGAATTAATG CTGAAAATCC TTACAAGAAC TTTATGCCAT CACCAGGTAA
    701  AATTGAGCAA TATCTTGCAC CAGGTGGATA TGGTGTTCGA ATAGAGTCAG
    751  CATGTTATAC TAATTATACG ATACCGCCAT ATTATGATTC GATGGTAGCG
45  801  AAATTAATCA TACATGAACC GACACGAGAT GARGCGATTA TGGSTGGCAT
    851  TCGTGCAC TA ARKGRWTTG TGGTTYTTGG GTATTGATAC AACTATTCCA

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5
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 15
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 25

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901 TTTCCATATT AAATTATTGA ATAACGGATA TATTTAGGAA GCGGTAAATT
951 TAATACAAAC TTTT TAGAAG CAAAATAGCA TTATTGAATG ATGAAAGGTT
1001 AATAGGAGGT CMATCCCMTG GTCAAAGTAA CTGATTATTC MAATTCMAAA
1051 TTAGGTAAAG TAGAAATAGC GCCAGAAGTG CTATCTGTTA TTGCAAGTAT
1101 AGCTACTTCG GAAGTCGAAG GCATCACTGG CCATTTTGCT GAATTAAAAAG
1151 AAACAAATTT AGAAAAAGTT AGTCGTAAAA ATTTAAGCCG TGATTAAAAA
1201 ATCGAGAGTA AAGAAGATGG CATATATATA GATGTATATT GTGCATTAAA
1251 ACATGGTGTT AATATTTCAA AAAC TGCAAA CAAAATTCAA ACGTCAATTT
1301 TTAATTCAAT TTCTAATATG ACAGCGATAG AACCTAAGCA AATTAATATT
1351 CACATTACAC AAATCGTTAT TGAAAAGTAA TGTACATACCT AATTCAGTAA
1401 TTAAATAAAG AAAAATACAA ACGTTTGAAG GAGTTAAAAA TGAGTCGTAA
1451 AGAATCCCGA GTGCAAGCTT TTCAAAC TTT ATTTCAATTA GAAATGAAGG
1501 ACAGTGATTT AACGATAAAT GAAGCGATAA GCTTTATTAA AGACGATAAT
1551 CCAGATTTAG ACTTCGAATT TATTCATTGG CTAGTTTCTG GCGTTAAAGA
1601 TCACGAACCT GTATTAGACG AGACAATTAG TCCTTATTTA AAAGATTGGA
1651 CTATTGCACG TTTATTAAAA ACGGATCGTA TTATTTTAAG AATGGCAACA
1701 TATGAAATAT TACACAGTGA TACACCTGCT AAAGTCGTAA TGAATGAAGC
1751 AGTTGAATTA ACAAACAAT TCAGTGATGA TGATCATTAT AAATTTATAA
1801 ATGGTGTATT GAGTAATATA AAAAAATAAA ATTGAGTGAT GTTATATGTC
1851 AGATTATTTA AGTGTTCAG CTTTAACGAA ATATATTAAA TATAAATTG
1901 ATCGACCTGC AGGCAT
  
```

Mutant: NT432

phenotype: temperature sensitivity

Sequence map: : Mutant NT432 is complemented by plasmid pMP500, which carries a 1.9 kb insert of wild-type *S.*

30 *aureus* genomic DNA. A partial restriction map is depicted in Fig. 84. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level similarities to the *pgsA* gene product, encoding CDP-diacylglycerol:glycerol-3-
 35 phosphate 3-phosphatidyltransferase (PGP synthase; EC 2.7.8.5) from *B. subtilis* (Genbank Accession No. D50064; published in Kontinen, V.P. et al. *FEBS lett.* 364 (1995) 157-160).

40 **DNA sequence data:** The following DNA sequence data represents the sequence generated by primer walking through clone pMP500, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below
 45 can be used to design PCR primers for the purpose of

amplification from genomic DNA with subsequent DNA sequencing.

clone pMP500

5

SEQ ID NO. 97

pMP500 Length: 1932 nt

```

      1  CGGGGATCCT CTAGAGTCGA TCCGTTTGGT GGTGGTTTTG GTTCTTTCGA
10      51  GTAAGTGTA  GGAGGCTATG AATTGARRAC GGTCGGTGAA GCGCTAAAAG
      101  GTANACGTGA AAGGTTAGGA ATGACTTYAA CAGAAATTAGA GCAACGTACT
      151  GGAATTAANC GTGAAATGCT AGTGCATATT GAAAATAATG AATTCGATCA
      201  ACTACCGAAT AAAAATTACA GCGAAGGATT TATTAGAAAA TATGCAAGCG
      251  TAGTAAATAT TGAACCTAAC CAATTAATTC AAGCTCATCA AGATGAAATT
15      301  CCATCGAACC AGAGCCGAAT GGGACGAAGT AATTACAGTT TTCAATAGAT
      351  AATAAAGACT TACGATTATA AGAGTAAATC AAAGANAGCC AATACAATTA
      401  TTAGTAATCA TGGGTTATTA CAGTTTAAAT AACTTTATTG TTATGGATCA
      451  TGTTAGTTTT AATATTTTAA CAGAAATAAA TTAGTGAGAA ATGAGGATGT
      501  TATAATGAAT ATTCCGAACC AGATTACGGT TTTTAGAGTT AGTGTTAATA
20      551  CCAGTTTTTA TATTGTTTGC GTTAGTTGAT TTTGGATTG GCAATGTGTC
      601  ATTTCTAGGA GGATATGAAA TAAGAATTGA GTTATTAATC AGTGGTTTTA
      651  TTTTATATAT GGCTTCCCTT AGCGATTTTG TTGATGGTTA TTTAGCTAGA
      701  AAATGGAATT TAGTTACAAA TATGGGGAAA TTTTGGATC CATTAGCGGA
      751  TAAATTATTA GTTGCAAGTG CTTTAATTGT ACTTGTCGAA CTAGGACTAA
25      801  CAAATTCTGT AGTAGCAATC ATTATTATTG CCAGAGAATT TGCCGTAAC
      851  GGTTCACGTT TACTACAAAT TGAACAAGGA TTCCGTAAGT TGCAGCTGGT
      901  CCAATTTAGG TWAAAWTWAA AACAGCCAGT TACTATGGTT AGCMAWTWAC
      951  TTGGTTGTTW ATTAAGKTGA TCCCATTGGG CAACATTGAT TGGTTTGTC
30      1001  ATTARGACAA ATTTTAATTA TAACATTGGC GTTATWTTTW ACTATCYTAT
      1051  CTGGTATTGA ATAACTTTAA TAAAGGTAGA GATGTTTTTA AACAAAAATA
      1101  AATATTTGTT TATACTAGAT TTCATTTTCA TATGGAATCT AGTTTTTTTA
      1151  ATCCCAATTT TAGAAATTAG CCACGCAATT GTTTATAATG ATATATTGTA
      1201  AAACAATATT TGTTCAATTT TTTAGGGAAA ATCTGTAGTA GCATCTGATA
      1251  CATTGAATCT AAAATTGATG TGAATTTTTA AATGAAATAC ATGAAAAAAT
35      1301  GAATTAAACG ATACAAGGGG GATATAAATG TCAATTGCCA TTATTGCTGT
      1351  AGGCTCAGAA CTATTGCTAG GTCAAATCGC TAATACCAAC GGACAATTTC
      1401  TATCTAAAGT ATTTAATGAA ATTGGACAAA ATGTATTAGA ACATAAAGTT
      1451  ATTGGAGATA ATAAAAACG TTTAGAATCA AGTGTAACGT CATGCGCTAG
      1501  AAAAATATGA TACTGTTATT TTAACAGGTG GCTTAGGTCC TACGAAAGAT
40      1551  GACTTAACGA AGCATAACAGT GGCCAGATT GTTGGTAAAG ATTTAGTTAT
      1601  TGATGAGCCT TCTTTAAAT ATATTGAAAG CTATTTTGAG GAACAAGGAC
      1651  AAGAAATGAC ACCTAATAAT AAACAACAGG CTTTAGTAAT TGAAGGTTCA
      1701  ACTGTATTAA CAAATCATCA TGGCATGGCT CCAGGAATGA TGGTGAATTT
      1751  TGAAAACAAA CAAATTATTT TATTACCAGG TCCACCGAAA GAAATGCAAC
45      1801  CAATGGTGAA AAATGAATTG TTGTCACATT TTATAAACCA TAATCGAATT
      1851  ATACATTCTG AACTATTAAG ATTTGCGGGA ATAGGTGAAT CTAAAGTAGA
      1901  AACAATATTA ATAGATCGAC CTGCAGGCAT GC

```

50

Mutant: NT435**phenotype:** temperature sensitivity

Sequence map: Mutant NT435 is complemented by plasmid
 5 pMP506, which carries a 3.2 kb insert of wild-type *S.*
aureus genomic DNA. A partial restriction map is depicted
 in Fig. 85. Database searches at the nucleic acid and
 (putative) polypeptide levels against currently available
 databases reveal strong peptide-level similarity from the
 10 left-most contig (shown below) to the *pdhA* gene product,
 encoding the E1-alpha subunit of pyruvate dehydrogenase,
 from *B. subtilis*. The right-most contig below demonstrates
 DNA sequence identity to the *pdhC* gene, encoding the E2
 chain of dihydrolipoamide acetyltransferase (EC 2.3.1.12),
 15 from *S. aureus* (Genbank Accession No. X58434). This
 Genbank entry also contains the *pdhB* gene upstream,
 encoding the E1-beta subunit of pyruvate dehydrogenase (EC
 1.2.4.1); since the pMP506 clone contains the region
 upstream of *pdhC*, it is predicted that the essential gene
 20 identified by mutant NT435 is *pdhB*. Further sequencing is
 currently underway to prove this assertion.

DNA sequence data: The following DNA sequence data
 represents the sequence generated from clone pMP506,
 25 starting with standard M13 forward and M13 reverse
 sequencing primers; the sequence contig will be completed
 via primer walking strategies. The sequence below can be
 used to design PCR primers for the purpose of amplification
 from genomic DNA with subsequent DNA sequencing.

30

clone pMP506**SEQ ID NO. 98**

pMP506.forward Length: 619 nt

35

40

45

```

1  ATTCGAGCTC GGTACCCGGG GATCCTCTAN AGTCGATCTT ACGGATGAAC
51  AATTAGTGGA ATTAATGGAA AGAATGGTAT GGAAGTCGTAT CCTTGATCAA
101 CGTTCATCTT CATTAAACAG ACAAGGACGT TTAGGTTTCT ATGCACCAAC
151 TGCTGGTCAA GAAGCATCAC AATTAGCGTC ACAATACGCT TTAGAAAAAG
201 AAGATTACAT TTTACCGGGA TACAGAGATG NTCCTCAAAT TATTTGGCAT
251 GGTTTACCAT TAACTGAAGC TTTCTTATTC TCAAGAGGTC ACTTCAAAGG
301 AAATCAATTC CCTGAAGGCG TTAATGCATT AAGCCCACAA ATTATTATCG
351 GTGCACAATA CATTCAAGCT GCTGGTGTTC GCATTTGCAC TTAAAAAACC
401 TTGGTAAAAA TGCAGTTGCA ATCACTTACA CTGGTTGACG GTGGTTCTTC
451 ACAAGGTTGA TTTCTACGAA GGTATTAACT TTGCAGCCAG CTTTATAAAG

```

```

501 CACCTGGCAA TTTTCCGTTA TTCAAAACAA TAACTATGCA ATTTCAACAC
551 CCAAGAANCA AGCNAACTGC TGCTGAAACA TTACTCAAAA ACCATTGCTG
601 TAGTTTTCTT GGTATCCAT

```

5 SEQ ID NO. 99

pMP506.reverse Length: 616 nt

```

1 CTTGCATGCC TGCAGGTCGA TCANCATGTT TAACAACAGG TACTAATAAT
51 CCTCTATCAG TGTCTGCTGC AATACCGATA TTCCAGTAAT GTTTATGAAC
10 101 GATTTACCA GCTTCTTCAT TGAATGAAGT GTTAAGTGCT GGGTATTTTT
151 TCAATGCAGA AACAAGTGCT TTAACAACAT AAGGTAAGAA TGTTAACTTA
201 GTACCTTGTT CAGCTGCGAT TTCTTTAAAT TTCTTACGGT GATCCCATAA
251 TGCTTGAACA TCAATTTTCAT CCATTAATGT TACATGAGGT GCAGTATGCT
301 TAGAGTTAAC CATTGCTTTC GCAATTGCTC TACGCATAGC AGGGATTTTT
15 351 TCAGTTGTTT CTGGGAAGTC GCCTTCTAAT GTTACTGCTG CAGGTGCTGC
401 AGGAGTTTCA GCAACTTCTT CACTTGTAGC TGAAGCAGCT GATTCAATTG
451 AAGCTGTTGG TGCACCACCA TTAAAGTATG CATCTACATC TTCTTTTGTA
501 ATACGACCAT TTTTACCAG ATCCAGAAAC TGCTTTAATG TTTAACACCT
551 TTTTCACGTG CGTTATTTAC TTACTGAAGG CATTGCTTTA AACAGTCTGT
20 601 TTTCATCTAC TTCCTC

```

25 **Mutant: NT437****phenotype:** temperature sensitivity

Sequence map: Mutant NT437 is complemented by plasmid pMP652, which carries a 3.1 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 86; no apparent restriction sites for EcoR I, Hind III, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal no significant similarities at this time. Current efforts are underway to complete the sequence contig and identify the essential gene contained in clone pMP652.

DNA sequence data: The following DNA sequence data represents the sequence generated from clone pMP652, starting with standard M13 forward and M13 reverse sequencing primers; the sequence contig will be completed via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

45

clone pMP652

SEQ ID NO. 100

pMP652.forward Length: 655 nt

```

5       1  GTACCGGGGA TCGTCACTTA NCCTCTCTAT TTCAATTTCA ACTTATTTTCG
       51  TCATCAAGTA TATGTGTTAT GCTTTTATAA CTTTGATTTC AATTCTATCA
      101  ATATCTGTGA CATTGATAAC ATCGGACATA CGGTCTTCTT GTAACTTTTT
      151  ATCCAATTCA AATGTATACT TTCCATAGTA TTTCTTTTTG ACTGTAATTT
      201  TTCCTGTACT CATTTTCACCG TAAAGACCAT AATTATCAAT AAGGTATTTT
10     251  CTTAATTTAA AATCAATCTC TTTCAATGAC ATCGCTTCTT TATCTATTTT
      301  AAATGGGAAA AAGTCATAAT CATATTCACC AGTATGATCT TCTTTAATAA
      351  CTCTTGCTTC TGCTATTAGG TCGACAGCTT TATCGTTTGC ACTCGTGATA
      401  CCCCCAATAG AGTACTTTGC ACCTTCAAAT CTCTTATCCT CATTAACGTA
      451  AAATATATTA AGAWTACGAW KKTACACCCG TATGATAATG TTTGCTTATC
15     501  TTTGCCAATT AAAGCAATAT TATTAACAGA ATTACCATCT ATGATATTCA
      551  TAAATTTAAT ACTTGGTTGA ATGAACTGG ATATAACCTG TCMCATTTTT
      601  AATATTCMAT ACTAGGTTGA ATWATAATAA GCTTTTAATT TTTKGCTATT
      651  TTCCC

```

20 SEQ ID NO. 101

pMP652.reverse Length: 650 nt

```

      1  GTCGACTCTA GAGGACTGCG TAATAACCTA TGAAAAATGA TATGAGCAAC
      51  GCCGCTCTGC TTTGCCGCAT ATACTAAAT TTCCACTTCA GGAATACGTT
25     101  TGAATGATGG ATGGATAATA CTTGGAATAA ACACAACGGT ATCCATTCCCT
      151  TTAAATGCTT CTACCATGCT TTCTTGATTA AAATAATCTA ATTGTCGAAC
      201  AGGAACTTTT CCGCGCCAAT CTTCTGGAAC TTTCTCAACA TTTCTAACAC
      251  CAATGTGAAA ATGATCTATG TGATTTGCAA TGGCTTGATT TGTAATATGT
      301  GTGCCTAAAT GACCTGTAGC ACCTGTTAAC ATAATATTCA TTCACTTCAT
30     351  CTCCTAATCT TTATATACAT AACATAATAC TTATTTGATG GTTTTCAAAA
      401  CATTTGATTT TATAAAAAAT TCTAATCTGT ATTTATTGTC GACGTGTATA
      451  GTAAATACGT AAATATTANT AATGTTGAAA ATGCCGTAAT GACGCGTTTT
      501  AGTTGATGTG TTTCACTAAT ATCATTGAAA ATTTTAATCA GGTACTACGA
      551  CAATATGAAG TCTGTTTTGT GTCTGAAAAT TTTACAGTTT TTAAATAATAA
35     601  AATGGTATAA GTTGTGATTT GGTTTAAAAA ANAATCTCGA CGGATAANAA

```

40 **Mutant: NT438****phenotype:** temperature sensitivity**Sequence map:** : Mutant NT438 is complemented by plasmid pMP511, which carries a 2.3 kb insert of wild-type *S.*

45 aureus genomic DNA. A partial restriction map is depicted in Fig. 87; no apparent restriction sites for EcoR I, Hind III, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level

similarities to the *nifS* gene product, encoding a protein involved in the response pathway for nitrogen assimilation, from *A. azollae* (Genbank Accession No L34879; published in Jackman, D.M. et al. *Microbiology* 141, pt.9 (1995) 2235-2244).

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP511, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP511

SEQ ID NO. 102

pMP511 Length: 2341 nt

```

20      1  CTTGCATGCC  TGCAGGTCGA  TCTTTATTAT  NATCTACACC  ACGTANCATT
      51  TCAACATGAC  CACGNTCATG  ACGATGTATG  CGTGCGTAAW  GTCCTGTKGY
     101  WACATAATCK  GCACCTAAAT  TCATCGCATG  ATCTAAAAAG  GCTTTAAACT
     151  TAATTTCTTT  ATWAMACATA  ACGTCTGGAT  TTGGAGTACG  ACCTTTTTTG
     201  TATTCATCTA  AGAAATACGT  AAAGACTTTA  TCCCAATATT  CTTTTTCAAA
     251  ATTAACAGCG  TAATACGGAA  TGCCAATTTG  ATTACACACT  TCAATAACAT
     301  CGTTGTAATC  TTCAGTTGCA  GTACATACGC  CATTTTCGTC  AGTGTCAATC
     351  CAGTTTTTCA  TAAATATGCC  AATGACATCA  TAACCTTGTT  CTTTTAAGAC
     401  GTGGGCTGTT  ACAGAACTAT  CTACACCGCC  TGACATACCA  ACGACAACAC
     451  GTTATATCTT  TATTTGACAA  TTATGACTCC  TCCTTAAATT  TAAAATATAT
     501  TTTATGAATT  TCAGCTACAA  TTGCATTAAT  TTCATTTTCA  GTAGTCAATT
     551  CGTTAAAACT  AAATCGAATC  GAATGATTTG  ATCGCTCCTC  ATCTTCGAAC
     601  ATTCATCTA  AAACATGCGA  CGGTTGTGTA  GAGCCTGCTG  TACATGCAGA
     651  TCCAGACGAC  ACATAGATTT  GTGCCATATC  CAACAATGTT  AACATCGTTT
     701  CAACTTCAAC  AAACGGAAAA  TATAGATTTA  CAATATGGCC  TGTAGCATCC
     751  GTCATTGAAC  CATTTAATTC  AAATGGAATC  GCTCTTTCTT  GTAATTTAAC
     801  TAAAAATTGT  TCTTTTAAAT  TCATTAAATG  AATATTGTTA  TCGTCTCGAT
     851  TCTTTTCTGC  TAATTGTAAT  GCTTTAGCCA  TCCCAACAAT  TTGCGCAAGA
     901  TTTTCAKTGC  CTAGCACGGC  GTTTCAATTC  TTGTTACCG  CCAAGTTGAG
     951  GATAATCTAG  TGTAACATGG  TCTTTAACTA  GTAATGCACC  GACACCTTTT
    1001  GGTCCGCCAA  ACTTATGAGC  AGTAATACTC  ATTGCGTCGA  TCTCAAATTC
    1051  GTCAAWCTTA  ACATCAAGAT  GTCCAATTGC  TTGAACCGCA  TCAACATGGA
    1101  AATATGCATT  TGTCTCAGCA  ATAATATCTT  GAATATCATA  AATTTGTTGC
    1151  ACTGTGCCAA  CTTTATTATT  TACAAACATA  ATAGATACTA  AAATCGTCTT
    1201  ATCTGTAATT  GTTTCTTCAA  GTTTGATCTA  AATCAATAGC  ACCTGTATCA
    1251  TCARCATCTA  GATATGTTTA  CATCAAAACC  TYCTCGCTCT  AATTGTTCAA
    1301  AAACATGTAA  CACAGAATGA  TGTTCATCT  TCGATGTGAT  AATGTGATTA
    1351  CCCAATTGTT  CATTTGCTTT  TACTATGCCT  TTAATTGCCG  TATTATTCTGA
  
```

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1401 TTCTGTTGCG CCACTCGTAA ATATAATTTC ATGTGTATCT GCACCAAGTA
1451 ATTGTGCAAT TTGACGTCTT GACTCATCTA AATATTTACG CGCATCTCTT
1501 CCCTTAGCAT GTATTGATGA TGGATTACCA TAATGCGAAT TGTAATTCGT
1551 CATCATCGCA TCTACTAACT TCAGGTTTTA CTGGTGTGGT CGCAGCATAA
5 1601 TCTGCATAAA TTTCCCATGT TTGGACAACT CCTCACAATT TTATCAATGT
1651 TCCAATAATA GCACCTTAAC ATACTATTTT TCTAACTTTT CTGTTTAACT
1701 TTATTTATAA TGTTTTTAAT TATATTTTAC CATTTTCTAC ACATGCTTTT
1751 CGATAGGCTT TTTTAAGTTT ATCGCTTTAT TCTTGTCTTT TTTATAAAAT
1801 TTAGTATTTG CAGATATTTT TTTATTTGTA AAATGTAACG TACTATTATT
10 1851 TTGGTTATGA GCAATTTAAT ATTTATCTGG TTATTCGGAT TGGTATACTT
1901 CTTATATCAT AAAAAAGGAA GGACGATATA AAAATGGCGG ATTAAATATT
1951 CAGCAKKRAA CCTTGTCCCT ATTCGAGAAG GTGAAGATGA ACAAACAGCA
2001 ATTAATAATA TGGTTAATCT CGCACACAT TTAGACGAAT TATCATATGA
2051 AAGATATTGG ATTGCTGAAC ACCATAACGC TCCCAACCTA GTAAGTTCAG
15 2101 CAACTGCTTT ATTAATTCAA CATACGTTAG AACATACGAA ACACATACGT
2151 GTAGGTTCTG GAGGCATCAT GTTACCCTAAT CATGCTCCAT TAATCGTTGC
2201 GGAACAATTT GGCACGATGG CAACATTATT TCCAAATCGT GTCGATTTAG
2251 GATTAGGACG TGCACCTGGA ACAGATATGA TGACCGCAAG TGCATTAAGA
2301 CGAGATCGAC TNTAGAGGAT CCCCGGGTAC CGAGCTCGAA T
20

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Mutant: NT462

25 **phenotype:** temperature sensitivity

Sequence map: : Mutant NT462 is complemented by plasmid pMP540, which carries a 2.0 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 88; no apparent restriction sites for EcoR I, Hind

30 III, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal limited peptide-level similarity to a transposase-like protein from *S. aureus*; the putative function of the ORF contained in clone pMP540

35 is unclear and will require further characterization.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP540, starting with standard M13 forward and M13

40 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

45

clone pMP540

SEQ ID NO. 103

pMP540 Length: 2026 nt

```

5      1  AAGGAAACCA CCAACACCTG CGCCAACCTAA ACCKCCTGTT AGTGCAGAAA
      51  TAACGCTAAT AGCCCCCGCA CCTAAAGCAG CTRKNGTTTT TGTATATGCA
     101  GAAGAAAGAT ATAATGTTGC AGTATCTTTA CCTGTTTCTA CATATTGAGT
     151  TTTACCCGCT CTCAATTGGT CTTACAGCTTT ATATTNTWT ATTCTTCTW
     201  TAGTAAATAT ATCTTCCRGT TTATAACCTT TTTTCTCAAG TTCATCAAAT
    10  251  AAATTTWGGT TACTCAAATA TATTACCTTT GCTTGAGAAT GGTCTAACTT
     301  ATCTTCAGCA TGAGCTACAT CTGAATTATA GAGATAATGA AATTGGACTA
     351  ACAATAATA CACCAGCAGC TRRTAATAAG AGATTTTAA TTCGTTTTTC
     401  ATTAGTTTCT TTTAGATGAT TTTTGTATTT AGATTTTCGT TAAACAGAAA
     451  CTAGATTTTT TCATGATCGA CCTATCTTTT GTCCAGATAC AGTGAGACCT
    15  501  TGTCATTTAA ATGATTTTTA ATTCGTCTTG TACCAGAGAC TTTTCTATTA
     551  GAATTAAGAA TATTTATGAC GGCTGTTCTA TGTTTGAATC ATCTTTAGTG
     601  ATTTTATTAT CTTTCTTTT TATAGAATCA TAATAGGTAC TTCTTAGTAT
     651  TATCAGGACT TTACACATTG NTGATACTGA ATANTGATGT GCATTCTTTT
     701  GAATGACTTC TATTTTTGCC CCATAATCAG CGCTACTTGC TTTAAAATAT
    20  751  CGTGCTCCAT TTTAAAATGT TGAACCTCTT TCGTAATTT AATCAGGTCT
     801  TTTTCTTCAT CCGATAAGTT ATCTTGGTGA TTGAATGTAC CCGTGTTTTG
     851  ATGTTGCTTT ATCCATTTTC CTACATTTTA TAACCGCCAT TTACAAACGT
     901  CGAAKGTGTG AAATCATACT CGCGTWTAA TTCATTCTTA GGCTTACCAT
     951  TTTTATATAA TCTAACCATT TGTAACCTAA ACTCTGAACT AAATGATCTT
    25 1001  CTTTCTCTTG TCATAATAAA ATCGCCTACT TTCTTAAATT AACAAATATCT
    1051  ATTCTCATAG AATTTGTCCA ATTAAGTGTA GACGATTCAA TCTATCAGCT
    1101  AGAATCATAT AACTTATCAG AAGCAAGTGA CTGTGCWTGT ATATTTGCCG
    1151  MTGATATAAT AGTAGAGTCG CCTATCTCTC AGGCGTCAAT TTAGACGCAG
    1201  AGAGGAGGTG TATAAGGTGA TGCTYMTTTT CGTTCAACAT CATAGCACCA
    30 1251  GTCATCAGTG GCTGTGCCAT TGCCTTTTTY TCCTTATTGG CTAAGTTAGA
    1301  CGCAATACAA AATAGGTGAC ATATAGCCGC ACCAATAAAA ATCCCTCAC
    1351  TACCGCAAAT AGTGAGGGGA TTGGTGTATA AGTAAATACT TATTTTCGTT
    1401  GTCTTAATTA TACTGCTAAT TTTTCTTTTT GTAAAATATG CAAGGTTTTA
    1451  AAGAGAAACA TCAAGAACTA AAAAAGGCTY TATGTCAAAT TGGACTGATG
    35 1501  CGTTCAATAT CCGAAGTTAA GCAACTAAAC ATTGCTTAAC TTCCTTTTTA
    1551  CTTTTTGGAG CGTAAAGTTT TGAACATAAT AATATTCGAT TGCGCAAATG
    1601  ATTGTAACCT CCATAACCAA AAGATGTACG TTTAATTAAT TTTATTTTGT
    1651  TATTTATACC TTCTAAAGGA CCATTTGATA AATTGTAATA ATCAATGGTT
    1701  ACACTATTAA AAGTGTCACA AATTCTTATG AATCTGGCAT AAACCTTGAA
    40 1751  TTAATAAAT AAGTAAGAAA ACCTCGGCAC TTTATCATTT TAATAGTGTC
    1801  GAGATTTTTA TAGATACTAC AAATATTTAT AACATAGTTA AACTCATCTA
    1851  ATGACTTATA TTTTTGTTTC ATCACAATAT GAACAATTAT TTATTGGACG
    1901  TATTTTGCTC TTTTTTTATT TCAGAACTG ACTTAGGATT TTTATTAAAT
    1951  TTTCTACCCA ATTCATCTGT ATAAGAAATA TCGGTATCAA ATTGAAAATC
    45 2001  ATCAACAGAT CGACCTGCAG GCATGC

```

50 Mutant: NT482

phenotype: temperature sensitivity

Sequence map: : Mutant NT482 is complemented by plasmid pMP560, which carries a 2.7 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 89. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong similarity at the peptide-level to the *folC* gene product, encoding folyl polyglutamate synthase (FGPS), from *B. subtilis* (Genbank Accession No. L04520; published in Mohan, S. et al., *J. Bacteriol.* 171 (1989) 6043-6051.)

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP560, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP560

SEQ ID NO. 104

25 pMP560 Length: 2736 nt

```

      1  TGCCTGCAGG TCGATCTTCT ATGTAAATAA TCAAATGACG TTTCTTCTAT
     51  AGATATAAAT TGATATASAA AACTAAAAAT ACAACTGCAA CTATAAGATA
    101  ACAATACTAC CAAATGACAA CCTCCTTATG TAAATTATAG TTAGTTATTA
    151  CCAAAATGTA AATATACACT ATTTTTC AAG AATTGAACCG CTTTTTCATT
    201  TAAATTTTTC AATATTGCTA AGCATAATTG ATGGATACTT TAACAACCCA
    251  TTACTGCTCG GCAAAATTAA TAATGGCAAG AAATTGAACC TTATAAACAC
    301  ATACGATTTA GAGCATAAAA AATAACCATG AAGCTCTACC TATTGATTAA
    351  ATARATTCTT CATGGCTATT TTAGTTTATG TTTTATAATG CTTCAAAGTC
    401  TAATTTTGAT TTAACCTTAC TTATGAAATA CAGACTACCG GTAATTACTA
    451  ATGTATCACC TTGATAATTT TTTATAAATT CAACGTAGTC ATCTACTAAT
    501  TGTATTTTCAT CATTTTCAAT ACTACCTACA ATTTCTTCTT TGC GTAACGC
    551  TTTCGGAAAA TCAAATTCAG TTGCATAAAA CGTATGCGCA ATTAACTTA
    601  AATGTTTGAC CATCTCGTTA ATCGGTTTTC CGTTTATTGC TGASAACAAA
    651  ATATCTACTT TTTCTTTATC ATGGTACTGT TTAATTGTAT CAATTAGAGC
    701  ATCTATACTC TCTGAATTAT GYGCGCCATC CAAAATGATT AAAGGYTTGT
    751  CATGCACCTG CTCAATACGT CCAGTCCAAC GAACTGATT C AATACCGTCT
    801  ATCATCTTAT TGAAATCTAA TTCAATTAAT CCTTGTTTAT TTAATTCAAT
    851  AAGAGCTGTT ATGGCTAATG CAGCAAWTTT GTTCTGATG TTTACCTAA
    901  CATGCTTAAA ATGATTGTTT CTAATTCATA ATCTTTATAA CGGTAAGTTA
    951  AATTCATCAT TTTGCGATAC AACAACAATT TCTCTATCTA ATTCAATGGC

```

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1001 TTTGCATGTT GTTCAATTGC GCGTTCACGA ACATATTTTA ATGCATCTTC
1051 ATTTTTTACA GCATATATCA CTGGAACKTT AGGSTTTATA ATCGCGCCYT
1101 TATCCCTAGC AATATCTAGA TAAGTACCAC CTAAAATATC TGTATGGTCT
1151 AGACCGATAC TAGTTAAGAT TGATAAAACC GGTGTAAAGA CATTGTGCGA
5 1201 ATCGTTCCTT ATACCCAATC CAGCCTCAAC AATGACAAA TCAACAGGAT
1251 GTATTTCAAC AAAATATAAA AACATCATCG CTGTGATTAT TTCGAATTCA
1301 GTTGCAAMMM CTAAATCTGT TTCAMSTTCC ATCATTTCAA TTAACGGTGT
1351 TAATACGTGA TACTAATTCT AACAATAGCG TCATTTGATA TTGGCAACAC
1401 CATTTAGRAT AATTCGTTCA TTAAATGTTT CAATAAACGG CGACGTAAAT
10 1451 GTACCTACTT CATAACCATT TTCAACTAAA GCTGTTCTAA GGTAAAGCAAC
1501 TGTAGAGCCT TTACCATTG TGCCACSKAC ATGAATACCC TTAATGWTAT
1551 TTTGAGGATT ATTAATTGT GCTAGCATCC ATTCCATACG TTTAACACCT
1601 GGTGATGATC CAAATTTAGT TCTTTCGTGT ATCCAATACA AGCTCTCTAG
1651 GTAATTCATT GTTACTAACT CCTATGCTTT TAATTGTTCA ATTCTTGCCCT
15 1701 TCACACCATC ATATTTTTCT TGATAATCTT GTTTTTTACG TTTTCTTCA
1751 TTTATAACCT TTTCAGGTGC TTACTTACA AAGTTTTCAT TAGAGAGCTT
1801 TTTATCTACT CTATCTAATT CGCTTTGAAG TTTAGCTAAT TCTTTTCCA
1851 AACGGCTGAT TTCCTTATCC ATATCAATTA GCCCTTCTTA ATGGTAATAC
1901 CCACTTTACC TGCAATTACA ACTGATGTCA TTGCTTTCTC AGGAATTTCC
20 1951 AACGTCAGTG CTAATATTTA AGGTACTAGG ATTACAGAAT TTGATTAAAT
2001 AATCTTTGTT TTGTGATAAA GTTGTTCAT TTTCTTTATC TTTAGCTTGA
2051 ATTAAAATAG GTATTTCTTT AGACAATGGC GTATTTACTT CTACACGTGA
2101 TTGTCTTACA GATTTAATGA TTTCAACAAG TGGTKGCATT GTTTGTAAAC
2151 TTTCTTCAA AATCAATGAT TCACGCACTT CTGGCCATGA AGCTTTAACA
25 2201 ATTGTGTCAC CTTCATGTGG TAACTTTGC CATATTTTCT CTGTTACAAA
2251 TGGCATGAAT GGATGTAGCA TTCTCATAAT ATTGTCTAAA GTATAACTCA
2301 ATACTGAACG TGTAACCTGT TTTGTCTCTT CATCATTACT ATTCAATTGA
2351 ATTTTACTCA TTTCAATGTA CCAATCACAG AAATCATCCC AAATGAAATT
2401 ATATAATGCA CGTCCAACCT CGCCGAATTC ATATTTGTCA CTTAAATCAG
30 2451 TAACTGTTGC AATCGTTTCA TTTAAACGTG TTAGAATCCA TTTATCTGCT
2501 AATGATAAGT TACCACTTAA ATCGATATCT TCAACTTTAA AGTCTTCACC
2551 GATATTCATT AAAGTGAAC GTGCCCCATT CCAGATTTTA TTGATAAAGT
2601 TCCACACTGA CTCAACTTTT TCAGTTGAGT ATCTTAAATC ATGTCCTGGA
2651 GATGAACCTG TTGCTAAGAA GTAACGCAAG CTATCAGCAC CGTATTCGTC
35 2701 AATAACATCC ATTGGATCGA CCTGCAGGCA TGCAAG

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40 Mutant: NT486

phenotype: temperature sensitivity

Sequence map: : Mutant NT486 is complemented by plasmid pMP567, which carries a 2.3 kb insert of wild-type *S. aureus* genomic DNA. A partial restriction map is depicted in Fig. 90; no apparent restriction sites for EcoR I, Hind III, BamH I or Pst I are present. Database searches at the nucleic acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level similarities to the *accA* gene product, encoding the alpha

subunit of acetyl-CoA-carboxylase carboxyl transferase (EC 6.4.1.2), from *B. stearothermophilus* (Genbank Accession No. D13095); this gene product forms part of an enzyme complex responsible for fatty acid biosynthesis and is thought to be essential.

DNA sequence data: The following DNA sequence data represents the sequence generated by primer walking through clone pMP567, starting with standard M13 forward and M13 reverse sequencing primers and completing the sequence contig via primer walking strategies. The sequence below can be used to design PCR primers for the purpose of amplification from genomic DNA with subsequent DNA sequencing.

clone pMP567

SEQ ID NO. 105

pMP567 Length: 2255 nt

```

20      1  CNCGNNAGCG  ANGTINGCCGA  GGATCCTCTA  GAGTCNATCG  GTTATCGGTG
      51  AAAAGATATG  TCGCATCATT  GATTACTGCA  CTGAGAACCG  TTTACCATTT
     101  ATTCTTTTCT  CTGCAAGTGG  TGGTGCACGT  ATGCAAGAAG  GTATTATTTT
     151  CTTGATGCAA  ATGGGTAAAA  CCAGTGTATC  TTTAAACGT  CATTCTGACG
     25  201  CTGGACTATT  ATATATATCA  TATTTAACAC  ATCCAACTAC  TGGTGGTGTA
     251  TCTGCAAGTT  TTGCATCAGT  TGGTGATATA  AATTAAAGTG  AGCCAAAAGC
     301  GTTGATAGGT  TTTGCAGGTC  GTCGAGTTAT  TGAACAGACA  ATAAACGAAA
     351  AATTGCCAGA  TGATTTCCAA  ACTGCAGAAT  TTTTATTAGA  GCATGGACAA
     401  TTGGATAAAG  TTGTACATCG  TAATGATATG  CGTCAAACAT  TGTCTGAAAT
     30  451  TCTAAAAATC  CATCAAGAGG  TGAATAAATA  ATGTTAGATT  TTGAAAAACC
     501  ACTTTTGTAA  ATTCGAAATA  AAATTGAATC  TTTAAAGAA  TCTCAAGATA
     551  AAAATGATGT  GGATTTACCA  AAGAAGAATT  TGACATGCCT  TGAARCGTCM
     601  TTGGRACGAG  AACTAAAAA  AATATATACA  AATCTAAAC  CATGGGATCG
     651  TGTGCAAATT  GCGCGTTTGC  AAGAAAGACC  TACGACCCTA  GATTATATTC
     35  701  CATATATCTT  TGATTCGTTT  ATGGAACACT  ATGGTGATCG  TAATTTTAGA
     751  GATGATCCAG  CAATGATTGG  TGGTATTGGC  TTTTAAATG  GTCGTGCTGT
     801  TACAGTYRTK  GGACAACAAC  GTGGAAAAGA  TACWAAAGAT  RATATTTATC
     851  GAAATTTTKG  GTATGGCGCA  TCCAGAAGGT  TATCGAAAAG  CATTACGTTT
     901  AATGAAACAA  GCTGAAAAAT  TCAATCGTCC  TATCTTTACA  TTTATAGATA
     40  951  CAAAAGGTGC  ATATCCTGGT  AAAGCTGCTG  AAGAACGTGG  ACAAAGTGAA
    1001  TCTATCGCAA  CAAATTTGAT  TGAGATGGCT  TCATTAAAAG  TACCAGTTAT
    1051  TGCGATTGTC  ATTGKYGAAG  GTGGCAGTGG  AGGTGCTCTA  GGTATTGGTA
    1101  TTGCCAATAA  AGYATTGATG  TTAGAGAATA  GTACTTACTC  TGWTATATCT
    1151  CCTGAAGGTG  CAGCGGCATT  ATTATGGAAA  GACAGTAATT  TGGCTAAAAT
     45  1201  YGCAGCTGAA  ACAATGAAWA  TTAGTCCCCA  TGATATTAAG  CAATTAGGTA
    1251  TTATAGATGA  TGYCATTTCT  GAACCACTTG  GCGGTGCACA  TAAAGATATT
    1301  GAACAGCAAG  CTTTAGCTAT  TAAATCAGCG  TTTGTTGCAC  AGTTAGATTC
    1351  ACTTGAGTCA  TTATCAACGT  GATGAAATTG  CTAATGATCG  CTTTGAAAAA
  
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5 1401 TTCAGAAATA TCGGTTCTTA TATAGAATAA TCAACTTGAG CATT TTTTATG
1451 TTAAATCGAT ACTGGGTTTT ACCATAAATT GAAGTACATT AAAACAATAA
1501 TTTAATATTT AGATACTGAA TTTTAACTA AGATTAGTAG TCAAAATTGT
1551 GGCTACTAAT CTTTTTTTAA TTAAGTTAAA ATAAAATTCA ATATTTAAAA
1601 CGTTTACATC AATTCAATAC ATTAGTTTTG ATGGAATGAC ATATCAATTT
1651 GTGGTAATTT AGAGTTAAAG ATAAATCAGT TATAGAAAGG TATGTCGTCA
1701 TGAAGAAAAT TGCAGTTTTA ACTAGTGGTG GAGATTCACC TGGAATGAAT
1751 GCTGCCGTAA GAGCAGTTGT TCGTACAGCA ATTTACAATG AAATTGAAGT
1801 TTATGGTGTG TATCATGGTT ACCAAGGATT GTTAAATGAT GATATTCATA
10 1851 AACTTGAATT AGGATCRAGT TGGGGATACG ATTCAGCGTG GAGGTACATT
1901 CTTGTATTCA GCAAGATGTC CAGAGTTTAA GGAGCAAGAA GTACGTAAAG
1951 TTGCAATCGA AACTTACGT AAAAGAGGGA TTGAGGGCCT TGTAGTTATT
2001 GGTGGTGACG GTAGTTATCG CGGTGCACAA CGCATCAGTG AGGAATGTAA
2051 AGAAATTCAA ACTATCGGTA TTCCTGGTAC GATTGACAAT GATATCAATG
15 2101 GTACTGATTT TACAATTGGA TTTGACACAG CATTAAATAC GATTATTGGC
2151 TTAGTCGACA AAATTAGAGA TACTGCGTCA AGTCACGCAC GAACATTTAT
2201 CATTGAAGCA ATGGGCCGTG ATTGTGGAGT CATCTGGAGT CGACCTGCTA
2251 GTCTT

II. Homologous Genes

As described above, the use of genes from other pathogenic bacterial strains and species which are homologous to the identified genes from *Staphylococcus aureus* is also provided. Such homologous genes not only have a high level of sequence similarity with the particular *S. aureus* genes, but also are functional equivalents. This means that the gene product has essentially the same biological activity. Therefore, the homologous genes are identifiable, for example, based on a combination of hybridization of all or a portion of one gene to its homologous counterpart, and the ability of the homologous gene to complement the growth conditional mutant of *S. aureus* under non-permissive conditions. The ability of the homologous gene to hybridize with sequences from the *S. aureus* gene provides that homologous gene using generally accepted and used cloning techniques. The ability of the homologous gene to complement a defective *S. aureus* gene demonstrates that the genes are essentially equivalent genes found in different bacteria.

Specific examples of methods for identifying homologous genes are described in Van Dijl et al., U.S. Patent 5,246,838, issued September 21, 1993. In addition to the direct hybridization methods for identifying and isolating homologous genes mentioned above, Van Dijl et al. describe the isolation of homologous genes by isolating clones of a host bacterial strain which contain random DNA fragments from a donor microorganism. In those clones a

specific host gene has been inactivated (such as by linkage with a regulatable promoter), and inserted homologous genes are identified by the complementation of the inactivated gene function. Homologous genes identified in this way can
5 then be sequenced.

If the function of the product of a specific host gene is known, homologous gene products can often be isolated (by assaying for the appropriate activity) and at least partially sequenced (e.g., N-terminal sequencing).
10 The amino acid sequence so obtained can then be used to deduce the degenerate DNA base sequence, which can be used to synthesize a probe(s) for the homologous gene. A DNA library from another microorganism is then probed to identify a clone(s) containing a homologous gene, and the
15 clone insert sequenced.

These and other methods for identifying homologous genes are well-known to those skilled in the art. Therefore, other persons can readily obtain such genes which are homologous to the genes corresponding to SEQ ID NO. 1-
20 105.

III. Evaluation of Gene as Therapeutic Target

A. General Considerations

While the identification of a particular bacterial
25 gene as an essential gene for growth in a rich medium characterizes that gene as an antibacterial target, it is useful to characterize the gene further in order to prioritize the targets. This process is useful since it

allows further work to be focused on those targets with the greatest therapeutic potential. Thus, target genes are prioritized according to which are more likely to allow identification of antibacterial agents which are:

- 5 1. Highly inhibitory to the target in relevant pathogenic species;
2. Cause rapid loss of bacterial viability;
3. Not have frequently arising resistance mechanisms;
4. Have high selectivity for the bacterial target and
10 little, or preferably no, effect on the related
 mammalian targets;
5. Have low non-specific toxicity to mammals; and
6. Have appropriate pharmacodynamic and physical
 properties for use as a drug.
- 15 Consequently, target genes are prioritized using a variety
 of methods, such as those described below.

B. Methods for Recognizing Good Targets

Essential genes can be characterized as either bactericidal or bacteriostatic. Earlier work with *Sal-*
20 *monella* mutants established that the bactericidal/bacteriostatic distinction was a characteristic of inhibition of the specific gene, rather than of a mutant allele, and could be characterized *in vitro*. (Schmid et al., 1989, *Genetics* 123:625-633.) Therefore, preferred
25 targets (high priority) are those which are highly bactericidal when inhibited, causing cell death. A subset of the bactericidal essential genes can be identified as

strongly bactericidal, resulting in rapid cell death when inhibited.

In *S. typhimurium*, inhibition of strongly bactericidal genes was shown to result in one of the following effects:

1. Cell lysis (such genes generally involved in cell wall biosynthesis);
2. Inhibition of protein synthesis;
3. DNA degradation; or
4. Entry into non-recoverable state involving cell cycle related genes.

In vivo switch

In addition to the prioritization of gene targets based on the observed *in vitro* phenotypes, further evaluation of a specific gene as a potential therapeutic target is performed based on the effects observed with loss of that gene function *in vivo*. One approach is the use of null mutants in which the mutant gene product is inactive at 37°C. In the case of essential genes for which temperature sensitive mutants were previously isolated, those mutant strains can be used in this evaluation if the gene product is essentially inactive at 37°C. If such a temperature sensitive mutant has not previously been isolated but a complementing clone of some growth conditional mutant is available, then the required null mutants can generally be isolated through the use of localized mutagenesis techniques (Hong and Ames, 1971, *Proc. Natl. Acad. Sci. USA* 68:3158-3162). The evaluation then involves the comparison of the

in vivo effects of the normal strain and the mutant strain.

The comparison involves determinations of the relative growth in vivo, relative bactericidal phenotype in vivo and differences in response in various infection models.

5 In addition to gene target evaluations using null mutant experiments, related evaluations can be performed using "in vivo switch" methods. Such methods allow control of the expression of a gene in vivo, and so provide information on the effects of inhibiting the specific gene
10 at various time points during the course of an infection in a model infection system. In effect, an in vivo switch provides a mimic of the administration of an inhibitor of a gene, even if such an inhibitor has not yet been identified.

 Such in vivo switch methods can be carried out by
15 using recombinant strains of a pathogenic bacterium, which carry a test gene transcriptionally linked with an artificially controllable promoter. One technique for doing this is to use the natural promoter for the test gene, and insert an operator site in a position so that transcription
20 will be blocked if a repressor molecule is bound to the operator. Expression of the repressor molecule is then placed under artificial control by linking the gene for the repressor with a promoter which can be controlled by the addition of a small molecule. For example, a β -lactamase
25 receptor/repressor/promoter system can be used to control expression of a lac repressor, which, in turn, will bind to a lac operator site inserted in the test gene. These DNA constructs are then inserted into bacteria in which the

endogenous copy of the test gene has been inactivated, and those bacteria are used in various infection models. Therefore, for this system, the test gene will be expressed prior to administration of a β -lactam. However, when a β -lactam with little or no intrinsic antibacterial activity (e.g., CBAP) is administered to an animal infected with the recombinant bacteria, the β -lactam induces production of lac repressor. The lac repressor molecule then binds to the lac operator, stopping (turning off) expression of the test gene.

The method can be extended by administering the β -lactam (or other appropriate controller molecule) at different times during the course of an infection, and/or according to different schedules of multiple dosing. Also, many different designs of *in vivo* switch may be used to provide control over the test gene. In general, however, such a method of target evaluation provides information such as:

1. a measure of the "cidalness" of the target gene following inhibition of that gene;
2. a benchmark against which to measure chemical inhibitors as they are identified, since the *in vivo* switch can mimic complete inhibition of the gene;
3. an estimate of the efficacy of inhibitor use at different time points in an infection process; and
4. an estimate of the efficacy of inhibitor use in various types of infections, in various *in vivo* environments.

Information of this nature is again useful for focusing on the gene targets which are likely to be the best therapeutic targets.

5 C. In vivo evaluation of microbial virulence and pathogenicity

Using gene target evaluation methods such as the null mutant and *in vivo* switch methods described above, the identified target genes are evaluated in an infection model system. (References herein to the use of animals or mammals
10 should be understood to refer to particular infection models. Other infection systems may be used, such as cell-based systems as surrogates for whole organism models, or systems to evaluate possible antimicrobial targets of pathogens of organisms other than animals (e.g., plants).
15 The criteria for evaluation include the ability of the microbe to replicate, the ability to produce specific exoproducts involved in virulence of the organism, and the ability to cause symptoms of disease in the animals.

The infection models, e.g., animal infection
20 models, are selected primarily on the basis of the ability of the model to mimic the natural pathogenic state of the pathogen in an organism to be treated and to distinguish the effects produced by activity or by loss of activity of a gene product (e.g., a switch in the expression state of the
25 gene). Secondly, the models are selected for efficiency, reproducibility, and cost containment. For mammal models, rodents, especially mice, rats, and rabbits, are generally the preferred species. Experimentalists have the greatest

experience with these species. Manipulations are more convenient and the amount of materials which are required are relatively small due to the size of the rodents.

Each pathogenic microbe (e.g., bacterium) used in these methods will likely need to be examined using a variety of infection models in order to adequately understand the importance of the function of a particular target gene.

A number of animal models suitable for use with bacteria are described below. However, these models are only examples which are suitable for a variety of bacterial species; even for those bacterial species other models may be found to be superior, at least for some gene targets and possibly for all. In addition, modifications of these models, or perhaps completely different animal models are appropriate with certain bacteria.

Six animal models are currently used with bacteria to appreciate the effects of specific genes, and are briefly described below.

20 1. Mouse Soft Tissue Model

The mouse soft tissue infection model is a sensitive and effective method for measurement of bacterial proliferation. In these models (Vogelman et al., 1988, *J. Infect. Dis.* 157: 287-298) anesthetized mice are infected with the bacteria in the muscle of the hind thigh. The mice can be either chemically immune compromised (e.g., cytoxan treated at 125 mg/kg on days -4, -2, and 0) or immunocompetent. The dose of microbe necessary to cause an

infection is variable and depends on the individual microbe, but commonly is on the order of 10^5 - 10^6 colony forming units per injection for bacteria. A variety of mouse strains are useful in this model although Swiss Webster and DBA2 lines are most commonly used. Once infected the animals are conscious and show no overt ill effects of the infections for approximately 12 hours. After that time virulent strains cause swelling of the thigh muscle, and the animals can become bacteremic within approximately 24 hours.

This model most effectively measures proliferation of the microbe, and this proliferation is measured by sacrifice of the infected animal and counting colonies from homogenized thighs.

2. Diffusion Chamber Model

A second model useful for assessing the virulence of microbes is the diffusion chamber model (Malouin et al., 1990, *Infect. Immun.* 58: 1247-1253; Doy et al., 1980, *J. Infect. Dis.* 2: 39-51; Kelly et al., 1989, *Infect. Immun.* 57: 344-350. In this model rodents have a diffusion chamber surgically placed in the peritoneal cavity. The chamber consists of a polypropylene cylinder with semipermeable membranes covering the chamber ends. Diffusion of peritoneal fluid into and out of the chamber provides nutrients for the microbes. The progression of the "infection" can be followed by examining growth, the exoproduct production or RNA messages. The time experiments are done by sampling multiple chambers.

3. Endocarditis Model

For bacteria, an important animal model effective in assessing pathogenicity and virulence is the endocarditis model (J. Santoro and M.E. Levinson, 1978, *Infect. Immun.* 19: 915-918). A rat endocarditis model can be used to
5 assess colonization, virulence and proliferation.

4. Osteomyelitis Model

A fourth model useful in the evaluation of pathogenesis is the osteomyelitis model (Spagnolo et al., 1993, *Infect. Immun.* 61: 5225-5230). Rabbits are used for these
10 experiments. Anesthetized animals have a small segment of the tibia removed and microorganisms are microinjected into the wound. The excised bone segment is replaced and the progression of the disease is monitored. Clinical signs, particularly inflammation and swelling are monitored.
15 Termination of the experiment allows histologic and pathologic examination of the infection site to complement the assessment procedure.

5. Murine Septic Arthritis Model

A fifth model relevant to the study of microbial
20 pathogenesis is a murine septic arthritis model (Abdelnour et al., 1993, *Infect. Immun.* 61: 3879-3885). In this model mice are infected intravenously and pathogenic organisms are found to cause inflammation in distal limb joints. Monitoring of the inflammation and comparison of
25 inflammation vs. inocula allows assessment of the virulence of related strains.

6. Bacterial Peritonitis Model

Finally, bacterial peritonitis offers rapid and predictive data on the virulence of strains (M.G. Bergeron, 1978, *Scand. J. Infect. Dis. Suppl.* 14: 189-206; S.D. Davis, 1975, *Antimicrob. Agents Chemother.* 8: 50-53). Peritonitis in rodents, preferably mice, can provide essential data on the importance of targets. The end point may be lethality or clinical signs can be monitored. Variation in infection dose in comparison to outcome allows evaluation of the virulence of individual strains.

A variety of other *in vivo* models are available and may be used when appropriate for specific pathogens or specific genes. For example, target organ recovery assays (Gordee et al., 1984, *J. Antibiotics* 37:1054-1065; Bannatyne et al., 1992, *Infect.* 20:168-170) may be useful for fungi and for bacterial pathogens which are not acutely virulent to animals. For additional information the book by Zak and Sande (EXPERIMENTAL MODELS IN ANTIMICROBIAL CHEMOTHERAPY, O. Zak and M.A. Sande (eds.), Academic Press, London (1986) is considered a standard.

It is also relevant to note that the species of animal used for an infection model, and the specific genetic make-up of that animal, may contribute to the effective evaluation of the effects of a particular gene. For example, immuno-incompetent animals may, in some instances, be preferable to immuno-competent animals. For example, the action of a competent immune system may, to some degree, mask the effects of altering the level of activity of the test gene product as compared to a similar infection in an

immuno-incompetent animal. In addition, many opportunistic infections, in fact, occur in immuno-compromised patients, so modeling an infection in a similar immunological environment is appropriate.

5 In addition to these *in vivo* test systems, a variety of *ex vivo* models for assessing bacterial virulence may be employed (Falkow et al., 1992, *Ann. Rev. Cell Biol.* 8:333-363). These include, but are not limited to, assays which measure bacterial attachment to, and invasion of,
10 tissue culture cell monolayers. With specific regard to *S. aureus*, it is well documented that this organism adheres to and invades cultured endothelial cell monolayers (Ogawa et al., 1985, *Infect. Immun.* 50: 218-224; Hamill et al., 1986, *Infect. and Imm.* 54:833-836) and that the cytotoxicity of
15 ingested *S. aureus* is sensitive to the expression of known virulence factors (Vann and Proctor, 1988, *Micro. Patho.* 4:443-453). Such *ex vivo* models may afford more rapid and cost effective measurements of the efficacy of the experiments, and may be employed as preliminary analyses
20 prior to testing in one or more of the animal models described above.

IV. Screening Methods for Antibacterial Agents

A. Use of Growth Conditional Mutant Strains

1. Hypersensitivity and TS Mutant Phenoprints

25 In addition to identifying new targets for drug discovery, the growth conditional mutants are useful for screening for inhibitors of the identified targets, even before the novel genes or biochemical targets are fully

characterized. The methodology can be whole-cell based, is more sensitive than traditional screens searching for strict growth inhibitors, can be tuned to provide high target specificity, and can be structured so that more biological
5 information on test compounds is available early for evaluation and relative prioritization of hits.

Certain of the screening methods are based on the hypersensitivity of growth conditional mutants. For example, conditionally lethal ts mutants having temperature
10 sensitive essential gene functions are partially defective at a semi-permissive temperature. As the growth temperature is raised, the mutated gene causes a progressively crippled cellular function. It is the inherent phenotypic properties of such ts mutants that are exploited for inhibitor
15 screening.

Each temperature sensitive mutant has secondary phenotypes arising from the genetic and physiological effects of the defective cellular component. The genetic defect causes a partially functional protein that is more
20 readily inhibited by drugs than the wild type protein. This specific hypersensitivity can be exploited for screening purposes by establishing "genetic potentiation" screens. In such screens, compounds are sought that cause growth inhibition of a mutant strain, but not of wild type, or
25 greater inhibition of the growth of a mutant strain than of a wild type strain. Such compounds are often (or always) inhibitors of the wild type strain at higher concentrations.

Also, the primary genetic defect can cause far-reaching physiological changes in the mutant cells, even in semi-permissive conditions. Necessity for full function of biochemically related proteins upstream and downstream of the primary target may arise. Such effects cause hypersensitivity to agents that inhibit these related proteins, in addition to agents that inhibit the genetically defective cellular component. The effects of the physiological imbalance will occur through metabolic interrelationships that can be referred to as the "metabolic web". Thus, in some cases, the initial genetic potentiation screen has the ability to identify inhibitors of either the primary target, or biochemically related essential gene targets.

With sufficient phenotypic sensors, a metabolic fingerprint of specific target inhibition can be established. Therefore, the mutant strains are evaluated to identify a diverse repertoire of phenotypes to provide this phenotypic fingerprint, or "phenoprint". These evaluations include hypersensitivities to known toxic agents and inhibitors, carbon source utilization, and other markers designed to measure specific or general metabolic activities for establishing a mutant phenoprint that will aid in interpretation of inhibitor profiles.

2. Determination of hypersusceptibility profiles

As an illustration of the hypersusceptibility profiles for a group of bacterial ts mutant strains, the minimal inhibitory concentrations (MICs) of various drugs

and toxic agents were determined for a set of *Salmonella typhimurium* temperature-sensitive essential gene mutants.

The MICs were measured by using a standard micro broth dilution technique following the recommendations of the National Committee for Clinical Laboratory Standards (1994). Bacteria were first grown in Mueller-Hinton broth at 30°C, diluted to 10⁵ cfu/ml and used to inoculate 96-microwell plates containing two-fold dilutions of antibiotics in Mueller-Hinton broth. Plates were incubated for 20h at a semi-permissive temperature (35°C) and the MIC was determined as the lowest dilution of antibiotic preventing visible growth.

A two-fold difference in the susceptibility level of the mutant strain compared to that of the parental strain is within the limits of the experimental variation and thus a ≥4-fold decrease in MIC was considered as a significant hypersusceptibility.

Example 1: Hypersensitivity of *S. aureus* *secA* mutants

The *secA* mutant strain NT65 was found to be more sensitive to compound MC-201,250. The MIC of this compound on NT65 is 0.62 µg/ml and that on the wild type strain is 50 µg/ml. The inhibitory effect of MC-201,250 on *secA* mutants increased as screening temperatures increased. Other *secA* mutants, which may represent different alleles of the gene, are also hypersensitive to this compound by varying degrees, examples are shown in Table 1 below.

Table 1	
Hypersensitivity of <i>secA</i> Alleles to MC201,250	
Strain	MIC ($\mu\text{g/ml}$)
NT65	0.62
NT328	1.25
NT74	2.5
NT142	5
NT15	10
NT67	10
NT122	10
NT112	20
NT368	20
NT413	20
Wild Type (WT)	50

Furthermore, introduction of the wild type *secA* allele into NT65 raised the MIC to the wild type level. These data suggest that the hypersensitivity results from the *secA* mutation in the mutants.

To further demonstrate that the hypersensitivity to MC-201,250 is due to the *secA* mutation that causes the temperature sensitivity, heat-resistant revertants, both spontaneous and UV-induced, were isolated from NT65 and tested for their responses to the compound. In a parallel experiment, MC-201250-resistant revertants were also isolated from NT65 and tested for their growth at nonpermissive temperatures. The results showed that revertants able to grow at 43°C were all resistant to MC-201250 at the wild type level (MIC=50 $\mu\text{g/ml}$) and vice versa. Revertants able to grow at 39°C but not at 43°C showed intermediate resistance to MC-201,250 (MIC=1.25-2.5 $\mu\text{g/ml}$ and vice versa. The correlation between the heat-

sensitivity and MC-201,250-sensitivity strongly suggests that the *secA* gene product may be the direct target for MC-201,250.

The benefits of using hypersensitive mutants for screening is apparent, as this inhibitor would have not been identified and its specificity on *secA* would have not been known if wild type cells rather than the mutants were used in whole cell screening at a compound concentration of 10 µg/ml or lower.

Example 2: Hypersensitivity of *S. typhimurium* gyr mutants

The specific hypersensitivity of temperature sensitive mutations in a known target to inhibitors of that target is shown in Figure 1 with the susceptibility profile of three ts *S. typhimurium* mutant alleles of the gyrase subunit A (*gyrA212*, *gyrA215* and *gyrA216*) grown at a semi-permissive temperature (35°C). The graph shows the fold-increases in susceptibility to various characterized antibacterial agents compared to that observed with the wild-type parent strain. The data demonstrate the highly specific hypersusceptibility of these mutants to agents acting on DNA gyrase. Susceptibility to other classes of drug or toxic agents is not significantly different from the parent strain (within 2-fold).

In addition, different mutant alleles show unique hypersensitivity profiles to gyrase inhibitors. Coumermycin inhibits the B-subunit of the gyrase, while norfloxacin,

ciprofloxacin, and nalidixic acid inhibit the A-subunit. One mutant shows hypersusceptibility to coumermycin (*gyrA216*), one to coumermycin and norfloxacin (*gyrA215*), and another to norfloxacin and ciprofloxacin (*gyrA212*). Note
5 that a mutation in the gyrase subunit A (*gyrA215*) can cause hypersensitivity to B-subunit inhibitors and could be used to identify such compounds in a screen. In addition, some *gyrA* mutant strains show no hypersensitivity to known inhibitors; potentially, these strains could be used to
10 identify novel classes of gyrase inhibitors. Overall these results show that a selection of mutated alleles may be useful to identify new classes of compounds that affect gyrase function including structural subunit-to-subunit interactions. Thus, use of the properties of the crippled
15 gyrase mutants in a screen provides a great advantage over biochemical-based screens which assay a single specific function of the target protein *in vitro*.

Example 3: Hypersensitivity profiles of
20 *Salmonella ts* mutants

Demonstration of the generalized utility of hypersensitive screening with the conditional lethal mutants has been obtained (Figure 2) by collecting hypersensitivity profiles from partly characterized *Salmonella* conditional *ts*
25 mutants. The table shows the increased susceptibility of the mutant strains to various characterized antibacterial agents compared to the wild-type parent strain. A two-fold difference in the susceptibility level is within the limits

of the experimental variation and thus a ≥ 4 -fold difference is significant.

A variety of hypersusceptibility profiles is observed among the *ts* mutants. These profiles are distinct from one another, yet mutants with related defects share similar profiles. The *parF* mutants, which have mutations closely linked to the *Salmonella* topoisomerase IV gene, are hypersusceptible to gyrase subunit B inhibitors (black circle), although these mutants are also susceptible to drugs affecting DNA or protein metabolism. Similarly, specificity within the hypersusceptibility profiles of two out of four *ts* mutants (SE7583, SE7587, SE5119 and SE5045) having possible defects in the cell wall biosynthesis machinery are also observed (mutants *dapA* and *murCEFG*, black diamond). The latter mutants are also susceptible to other agents and share their hypersusceptibility profile with a mutant having a defect in the incorporation of radioactive thymidine (SE5091).

Thus, the hypersensitivity profiles actually represent recognizable interrelationships between cellular pathways, involving several types of interactions as illustrated in Fig. 3. The patterns created by these profiles become signatures for targets within the genetic/metabolic system being sensitized. This provides a powerful tool for characterizing targets, and ultimately for dereplication of screening hits. The hypersusceptibility profiles have been established for 120 *Salmonella* and 14

Staphylococcus aureus ts mutants with a selection of 37 known drugs or toxic agents

The growth conditional mutants are also used in gene sensor methodology, e.g., using carbon utilization profiles. Ts mutants fail to metabolize different carbon sources in semi-permissive growth conditions. The carbon sources not utilized by a specific mutant or group of mutants provide additional phenotypes associated with the crippled essential function. Moreover, some of these carbon source markers were also not used by the wild type strain exposed to sub-MIC concentrations of known drugs affecting the same specific cellular targets or pathways. For example, a sublethal concentration of cefamandole prevented the *Salmonella* wild type parent strain from metabolizing the same carbon source that was not used by either the *dapA* or the *murCEFG* mutant.

In combination, interrelationships within and between essential cellular pathways are manifested in hypersensitivity and biosensor profiles that together are employed for highly discriminatory recognition of targets and inhibitors. This information provides recognition of the target or pathway of compound action.

B. Screening Strategy and Prototypes

1. Strain Validation and Screening Conditions

Hypersensitive strains (not growth conditional) have been successfully used in the past for discovery of new drugs targeting specific cellular pathways. (Kamogashira and Takegata, 1988, *J. Antibiotics* 41:803-806; Mumata et

al., 1986, *J. Antibiotics* 39:994-1000.) The specific hypersensitivities displayed by ts-conditional mutants indicates that use of these mutants in whole cell screening provides a rapid method to develop target-specific screens for the identification of novel compounds. However, it is beneficial to eliminate mutants that will not be useful in semi-permissive growth conditions. Such mutant alleles may have nearly wild type function at the screening assay temperature. The simplest method for validating the use of ts mutants is to select those which show a reduced growth rate at the semi-restrictive growth temperature. A reduced growth rate indicates that the essential gene function is partially defective. More specific methods of characterizing the partial defect of a mutant strain are available by biochemical or physiological assays.

2. Multi-Channel Screening Approach

The phenoprint results above, demonstrate that ts mutants show specific hypersusceptibility profiles in semi-permissive growth conditions. As a screening tool, the mutant inhibition profile characterizes the effects of test compounds on specific bacterial pathways. Because the mutants are more sensitive than wild type strains, compounds with weak inhibition activity can be identified.

An example of a multi-channel screen for inhibitors of essential genes is shown in Fig. 4. In this screen design, one plate serves to evaluate one compound. Each well provides a separate whole-mutant cell assay (i.e., there are many targets per screening plate). The assays are

genetic potentiation in nature, that is, ts-hypersensitive mutants reveal compounds that are growth inhibitors at concentrations that do not inhibit the growth of the wildtype strain. The profile of mutant inhibition provides insight into the compound's target of inhibition. The ts mutants are grouped by their hypersensitivity profiles to known drugs or by their related defective genes. The figure illustrates the hypothetical growth inhibition results (indicated by "-") that would be obtained with a new antibacterial agent targeting DNA/RNA metabolism.

Different multi-channel screen designs can fit specific needs or purposes. The choice of a broadly-designed screen (such as in Fig. 4), or one focused on specific cellular pathways, or even specific targets can be made by the appropriate choice of mutants. More specific screen plates would use mutants of a specific gene target like DNA gyrase, or mutants in a specific pathway, such as the cell division pathway.

The use of the 96-well multi-channel screen format allows up to 96 different assays to characterize a single compound. As shown in Fig. 5, this format provides an immediate characterization or profile of a single compound.

The more traditional format, using up to 96 different compounds per plate, and a single assay can also be readily accommodated by the genetic potentiation assays.

In comparing the two formats, the multi-channel screen format is generally compound-focused: prioritization of compounds run through the screen will occur, as decisions

are made about which compounds to screen first. Each plate provides an immediate profile of a compound. The more traditional format is target-focused: prioritization of targets will occur, as decisions are made about the order of targets or genetic potentiation screens to implement.

In a preferred strategy for screening large compound libraries, a "sub-library" approach is taken. In this approach, the compound library is divided into a number of blocks or "sub-libraries". All of the selected mutants are screened against one block of the compounds. The screen is carried out in 96-well plates and each plate serves to test 80 compounds (one compound per well) on one mutant strain. After a block of compounds are screened, the mutant collection is moved on to test the next compound block.

The advantage of this strategy is that the effect of a compound on all the selected mutant strains can be obtained within a relatively short time. This provides compound-focused information for prioritization of compounds in follow-up studies. Since this strategy has only one mutant instead of many mutants on a plate, cross contamination between different strains and the testing of different mutants at different temperatures (or with other changes in assay conditions) are no longer problems. Moreover, this strategy retains the same compound arrangement in all compound plates, thus saving time, effort and compounds as compared to screening one compound

against many mutants on one plate, for compound focused analysis.

Example 4: Prototype Screening Protocol

S. aureus bacterial cells from pre-prepared frozen stocks are diluted into Mueller-Hinton (MH) broth to an OD600 of about 0.01 and grown at 30°C till OD600=0.5. Cells are diluted 1,000-fold into MH broth and 50 µl is added to each well of 96-well plates to which 40 µl of MH broth and 10 µl of test compound (varying concentrations) are added. No-compound wells with or without cells are included as controls. The total volume in each well is 100 µl. The plates are incubated at an appropriate screening temperature for 20 hr and OD600 are read. The effect of each compound on a mutant is measured against the growth control and % of inhibition is calculated. Wild type cells are screened at the same conditions. The % of inhibition of a compound on a mutant and that on the wild type cell are compared, and compounds that show higher inhibition on the mutant than on the wild type are identified.

20 3. Screening Method Refinement

Certain testing parameters for the genetic potentiation screening methods can significantly affect the identification of growth inhibitors, and thus can be manipulated to optimize screening efficiency and/or reliability. Notable among these factors are variable thermosensitivity of different ts mutants, increasing hypersensitivity with increasing temperature, and

"apparent" increase in hypersensitivity with increasing compound concentration.

a. Variable Thermosensitivity

To use *S. aureus* ts mutants in genetic
5 potentiation screening, the growth of these mutants at
different temperatures were measured to determine screening
temperatures for each of these mutants. The results showed
that different ts mutants have quite different maximum
growth temperatures (MGT). The MGTs of some mutants are as
10 high as 39°C, while those of others are 37°C, 35°C, 32°C or
even 30°C (Fig. 6). Furthermore, different mutants that
have mutations in the same gene may have quite different
MGTs, as illustrated in Fig.7 for several *polC* mutants.
Thus, different screening temperatures should be chosen for
15 these mutants in order to accommodate the different growth
preferences.

b. Raising screening temperature makes ts
mutants more sensitive to certain compounds

To demonstrate that the ts mutants are more
20 sensitive to potential inhibitors at elevated temperature,
the effect of different temperatures on the sensitivity of
several ts mutants to a subset of compounds was examined.
Figure 8 shows the inhibitory effect of 30 compounds on
mutant NT99 at 3 different temperatures, 32°C, 35°C, and
25 37°C. Most of these compounds showed increasing inhibitory
effect as temperature increased from 32° to 35°C then to
37°C. Consequently, more hits were identified at 37°C (Fig.
9). In fact, all the hits identified at 32°C and 35°C were

included in the 37°C hits. On the other hand, little difference was observed when the compounds were tested on wild type cells at the same three different temperatures (data not shown).

5 The temperature effect as mentioned above can be used to control hit rates in the screening. Higher screening temperature can be used to produce more hits for mutants that have low hit rates. Similarly, if a mutant shows a very high hit rate, the number of hits can be
10 reduced by using lower screening temperatures to facilitate hit prioritization.

c. Increasing compound concentrations
affect apparent hypersensitivity

15 The concentration of compounds used in the screening is an important parameter in determining the hit rates and the amount of follow-up studies. The concentration of 10 µg/ml has been used in piloting screening studies. To examine whether screening at lower concentrations can identify a similar set of hits, 41
20 compounds previously scored as hits were screened against their corresponding hypersensitive mutants at lower concentrations. Results in Fig. 10 showed that the number of compounds to which the target mutants were still
25 hypersensitive (≥80% inhibition) decreased as the screening concentrations decreased. At 2µg/ml, only 20 out of 41 hit compounds were able to be identified as hits that inhibit the mutants by ≥80%, and at 1 µg/ml only 11, or 27%, of the compounds still fell into this category. These data suggest

that screening at concentrations $<2 \mu\text{g/ml}$ may miss at least half of the hits that would be identified at $10 \mu\text{g/ml}$. On the other hand, screening at concentrations higher than $10 \mu\text{g/ml}$ may result in large number of low
5 quality hits and create too much work in hit confirmation and follow-up studies. At $10 \mu\text{g/ml}$, a hit may appear as a growth inhibitor for both the mutant and wild type strains. This should not be a major problem since lower concentrations of the compound can be tested in the follow-
10 up studies to differentiate its effect on the mutant and the wild type.

4. Evaluation of uncharacterized known growth inhibitors

In addition to testing known inhibitors of
15 cellular pathways, uncharacterized growth inhibitors identified in other whole-cell screens were also evaluated using temperature sensitive mutants. These growth inhibitors had uncharacterized targets of action. These compounds were previously shown to cause some growth
20 inhibition of the *S. aureus* strain 8325-4 at 5 mg/ml . The compounds were subsequently tested using a range of concentrations against a collection of *S. aureus* ts mutants (all derived from *S. aureus* 8325-4), to determine the MIC values, relative to wild type. Figure 12 summarizes the
25 data generated using 52 *S. aureus* ts mutants and 65 growth inhibitor compounds (47 compounds not shown). The table reports the fold-increase in susceptibility of the ts mutants compared with the wild-type parent strain; values

within two-fold of wildtype have been left blank in the table for ease of identifying the significant hypersensitive values.

The effects of the 65 test compounds on the ts mutants were mostly selective: for most compounds, a limited number of mutants were hypersensitive. Approximately one-third of all compounds showed identical inhibition of mutant and wild type strains (*i.e.*, no mutants were hypersensitive to these compounds). Two compounds in Figure 12 showed strong inhibitory effects on about 50% of the mutants tested (compounds 00-2002 and 00-0167). Two additional compounds showed identical inhibition profiles (compounds 30-0014 and 20-0348, Figure 12). A preliminary analysis of these profiles is provided below.

The genetic basis of the hypersensitivity has been substantiated by two criteria. First, one compound (10-0797) strongly inhibited two mutants (NT52 and NT69) that both affect the same gene. Secondly, complementation of the temperature sensitive phenotype of these mutants resulted in loss of hypersensitivity.

Furthermore, the two compounds that had identical inhibition profiles (30-0014 and 20-0348) have very similar structures (Figure 11). Thus, the hypersensitivity profile provides a pattern that allows recognition of compounds with similar targets of action, even when the target may be poorly defined. The strong similarity in the structures of these compounds makes their common target of action likely.

Based on the mutants that were inhibited (*secA*, *dnaG*, and

3 uncharacterized mutants) the target of action of these compounds is not yet defined.

It is preferable to perform a screen of the uncharacterized inhibitors against a larger number of ts mutants. This screen employs preset compound concentrations and obtains the mutant inhibition profile for each compound. Computing the difference in the relative growth of parent and mutant strains in the presence of compounds provides a compound profile similar to that obtained by the MIC determinations of the first screen above.

A wide range of test compounds can be screened. Test compounds that are inhibitory for the wild type parent strain at the pre-selected concentration in the first screening run are retested at a lower concentration to generate an inhibition profile. Data analysis from the screens described above showed that a significant growth reduction of mutant strains compared to the parent strain in the presence of the test compounds is a reasonable indicator of selective compound activity.

Further, compounds for testing can include compounds that show no growth inhibition of the wild type strain. The hypersensitivity of the mutant strains provides the ability to identify compounds that target an essential cellular function, but which lack sufficient potency to inhibit the growth of the wild type strain. Such compounds are modified using medicinal chemistry to produce analogs with increased potency.

The grid shown in Figure 13 represents different mutant inhibition profiles anticipated from screening of growth inhibitors, where "x" denotes inhibition of a particular mutant by a particular compound at concentrations much lower than for wildtype.

This grid shows compounds that cause growth inhibition of more than one mutant (compounds A,C,D,E), compounds that inhibit just one mutant (compounds B,F) and one compound that inhibits no mutants (compound G). In addition, this profile identifies mutants inhibited by no compound (mutant 8), a single compound (mutants 1,6,7), and several compounds (mutants 2,3,4,5). In the preliminary screens described above, compounds were identified that fit some of these anticipated inhibition profiles (see Fig. 14).

In the preliminary screen, compounds that inhibit the growth of the wild type strain were diluted to a point where growth inhibition of wild type no longer occurred. In this situation, only mutants that are hypersensitive to a particular compound will fail to grow. Thus, even compounds considered "generally toxic" should show some specificity of action, when assayed with the hypersensitive mutant strains.

In the simplest interpretation, compounds that cause growth inhibition inhibit the function of one essential macromolecule. Some compounds may specifically inhibit more than one target macromolecule. However, since one of the targets will be most sensitive to inhibition, one target can be considered the primary target. Thus, a

one-to-one correspondence between inhibitors and targets can be established. However, both the data, and less simplistic reasoning provide exceptions to the simple one-to-one relationship between targets and inhibitors. Further
5 analysis and understanding of the complicating effects is necessary to make full use of the data. Some of the complicating effects are discussed below.

a. Compounds that affect many mutants.

Certain compounds, such as detergents that target membrane
10 integrity, or DNA intercalators, will have "general", rather than specific targets. These "general targets" are not the product of a single gene product, but rather are created by the action of many gene products. Thus, in analyzing hypersensitivity profiles, compounds that affect many
15 mutants may indicate action on a "general target". The profiles of known membrane active agents, and intercalators will provide information to recognize uncharacterized compounds with similar effects.

Compounds that cause growth inhibition of more
20 than one mutant may also arise when the affected mutants are metabolically related. These mutants may affect the same gene, or the same biochemical pathway. For example, mutants defective in one of many cell wall biosynthetic steps may show hypersensitivity to compounds that inhibit any of these
25 steps. Evidence for this type of effect was observed in the hypersensitivity patterns of known inhibitors (see Figure 2). This concept can be broadened to include effects caused by the "metabolic web", in which far-reaching consequences

may arise through characterized and uncharacterized interrelationships between gene products and their functions.

Overall, the hit rate was high when we considered
5 all compounds that were more active on mutants than on the parent strain. The histogram in Figure 14 shows the hit rate for compounds that affected one, two, three, or more than three mutants in our prototype screen. The large number of compounds that affected more than three different
10 mutants was at least partly explained by the greater potency of this group of compounds. Figure 15 illustrates the potency of some of the hits found in the screen as evaluated by the MIC obtained for the parent strain *S. aureus* 8325-4.

In the prototype screen, compounds affecting more
15 than 3 mutants were generally more potent but some may also be considered broadly toxic. The columns identified by an asterisk in Figure 15 represent 3 out of 4 compounds that were also shown to be inhibitors of *Salmonella typhimurium* in another whole cell screen. Consequently, only the most
20 hypersusceptible strain of a group of mutants affected by the same compound should be considered as the primary target. However, the entire mutant inhibition profile of a specific compound is very useful and should be considered as its actual fingerprint in pattern recognition analysis.

25 b. Compounds that affect few (or no) mutants. Since all compounds assayed in the preliminary screen inhibit the growth of the wild type strain to some degree (initial basis of pre-selection), such compounds

indicate that the mutant population is not sufficiently rich to provide a strain with a corresponding hypersensitive target.

c. Mutants affected by many compounds.

5 Another complication of the simple one-to-one compound/target relationship will arise because of mutants that are inhibited by many different compounds. The relative number of compounds (% hits) that inhibited the growth of each mutant in the *S. aureus* pilot is shown in
10 Figure 16. Several mutants were affected by many compounds.

Several distinct causes of this are apparent. First, some mutants may have defects in the membrane/barrier that cause hyperpermeability to many different compounds. Such mutants will have higher intracellular concentrations of many
15 compounds, which will inhibit metabolically unrelated targets. Other mutants may have defects that have far-reaching consequences, because their gene products sit at critical points in the metabolic web. Still other mutants may have specific alleles that are highly crippled
20 at the assay temperature. For these mutants, the metabolic web consequences are large because the specific allele has created a highly hypersensitive strain.

d. Mutants affected by few or no compounds.

For the mutants that were hypersusceptible to fewer
25 compounds, it is possible that their mutations affect a limited metabolic web, that mutations provide a true specificity that was yet not revealed by any compound, or that these mutants have nearly full activity at the assay

temperature. This analysis stresses the importance of strain validation as indicated above.

In interpreting these patterns, the number of mutants screened and the total number of targets are also
5 important variables. These numbers provide a simple probabilistic estimate of the fraction of the compounds that should have a one-to-one correspondence with a mutant target in the sample that was screened.

6. Prioritization of Hits and Downstream
10 Development

The early steps in a multi-channel genetic potentiation screen include the following:

- Pre-selection of mutant strains for screening
- 15 • Pre-selection of desired test compounds based on structural features, biological activity, etc. (optional)
- Testing of the chosen compounds at a pre-determined concentration, preferably in the range 1-10 µg/ml.
- 20 • Analysis of inhibitory profiles of compounds against the mutant population and selection of interesting hits
- Confirmation of the selective inhibitory activity of the interesting hits against specific mutants
- 25 • Secondary evaluation of prioritized hits.

Genetic potentiation assays provide a rapid method to implement a large number of screens for inhibitors of a

large number of targets. This screening format will test the capacity of rapid high-throughput screening. The capability to screen large numbers of compounds should generate a large number of "hits" from this screening.

5 Limitations in downstream development through medicinal chemistry, pharmacology and clinical development will necessitate the prioritization of the hits. When large numbers of hits are available, each with reasonable *in vitro* activity, prioritization of hits can proceed based on
10 different criteria. Some of the criteria for hit characterization include:

- chemical novelty
- chemical complexity, modifiability
- 15 • pharmacological profile
- toxicity profile
- target desirability, ubiquity, selectivity

Secondary tests will be required not only for the
20 initial evaluation of hits, but also to support medicinal chemistry efforts. While the initial genetic potentiation tests will be sufficient to identify and confirm hits, selection of hits for further development will necessitate establishment of the specific target of action. Equipped
25 with the gene clones, selection of resistant alleles provides early evidence for the specific target. Subsequent efforts to establish a biochemical assay for rapid, specific and sensitive tests of derivative compounds will be aided by

the over-expression and purification of the target protein, sequence analysis of the ORF to provide early insight into novel target function, as well as a variety of physiological and biochemical tests comparing the mutant and wild type strain to confirm the novel target function, and aid in the establishment of biochemical assays for the targets.

7. Identification of Specific Inhibitors of Gene Having Unknown Function

In a piloting screening study, a number of compounds were identified as inhibitors for mutants with mutations located in open reading frames whose functions are not known. Some of the open reading frames have been previously identified in other bacteria while others show little homology to the current Genbank sequence collection. An example is mutant NT94, whose complementing clones contain an open reading frame that is homologous to a spoVB-like gene in *B. subtilis*. While the function of the gene is not clear in either *B. subtilis* or *S. aureus*, NT94 is hypersensitive to many compounds tested, as illustrated in Table 2 below.

Table 2			
Hit Rates in Genetic Potentiation Screen			
Number of mutants n, on which cmpds active		Confirmed Hits	
		39 mutants	NT94
n = 1 or 2	Average hit rate	0.03%	1.06%
	Hit rate range among mutants	0 - 0.31%	
n => 3	Average hit rate	0.17%	1.39%

Table 2			
Hit Rates in Genetic Potentiation Screen			
	Hit rate range among mutants	0 - 0.72%	

In fact, NT94 had the highest hit rate among the 40 mutant strains tested. Among the NT94 hits, 4 compounds share similar chemical structures (Figs. 19A-D). The MICs of these compounds on NT94 are 0.25-2 µg/ml, which are 16-256 fold lower than those on the wild type cells (32-64 µg/ml). The similarity in the compound structures suggests a common and specific mechanism of the inhibitory effect on NT94.

Furthermore, the hypersensitivity to these compounds can be abolished by introducing 2 or more copies of the wild type gene into NT94. A correlation between the copy number of the wild type gene and the tolerance to the compounds has been observed. Cells with 2 copies of the wild type gene are slightly more resistant (2-fold increase in MIC) to MC-207,301 and MC-207,330 than the wild type cells which has one gene copy; cells carrying complementing plasmids (about 20-50 copies per cell) are much more resistant (8-16 fold increase in MIC). Such a gene dosage effect further suggests that either the gene product itself or its closely related functions of the open reading frame affected in NT94 is the target of the hit compounds.

8. Multi-Channel Screen Advantages

As depicted by the *S. aureus* example shown above, multi-channel screen design rapidly leads to the identification of hits and provide some of the necessary

specificity information to prioritize compounds for further evaluation. Figure 17 illustrates the advantages of a genetic potentiation approach as the basis of a screen design.

5 Overall, an approach using whole-cell genetic potentiation of ts mutants includes the selectivity of the biochemical screens (it is target-specific, or at least pathway-specific) and it is more sensitive than traditional screens looking for growth inhibitors due to the
10 hypersensitive nature of the mutants. This genetic potentiation approach also provides a rapid gene-to-screen technology and identifies hits even before the genes or biochemical targets are fully characterized.

9. Alternatives to Ts Hypersensitivity Screening

15 There are a number of additional strategies that can be undertaken to devise target-based whole cell screens, as well as binding or biochemical type screens. In order to implement these strategies, knowledge of the existence of the gene, the DNA sequence of the gene, the hypersensitivity
20 phenotype profile, and the conditional mutant alleles will provide significant information and reagents. Alternative strategies are based on:

- over- and under-expression of the target gene
- 25 • dominant mutant alleles
- hypersensitive mutant alleles

a. Over- and Under-expression of Target

Genes. There are numerous examples of over-expression phenotypes that range from those caused by 2-fold increases in gene dosage (Anderson and Roth, 1977, *Ann. Rev. Microbiol.* 31:473-505; Stark and Wahl, 1984, *Ann. Rev. Biochem.* 53:447-491) to multi-fold increases in dosage which can be either chromosomal-encoded (Normark et al., 1977, *J. Bacteriol.* 132:912-922), or plasmid-encoded (Tokunaga et al., 1983, *J. Biol. Chem.* 258:12102-12105). The phenotypes observed can be analog resistance (positive selection for multiple copies, negative selection for inhibition phenotype) or growth defects (negative selection for multiple copies, but positive selection for inhibition phenotype).

Over-expression can be achieved most readily by artificial promoter control. Such screens can be undertaken in *E. coli* where the breadth of controllable promoters is high. However, this method loses the advantage gained by whole cell screening, that of assurance that the compound enters the pathogen of interest. Establishing controllable promoters in *S. aureus* will provide a tool for screening not only in *S. aureus* but most likely in other Gram-positive organisms. An example of such a controllable promoter is shown by controlled expression of the *agr* P3 promoter in the *in vivo* switch construction.

b. Dominant alleles. Dominant alleles can provide a rich source of screening capabilities. Dominant alleles in essential genes will prevent growth unless

conditions are established in which the alleles are non-functional or non-expressed. Methods for controlled expression (primarily transcriptional control) will provide the opportunity to identify dominant mutant alleles that prevent cell growth under conditions of gene product expression.

Equally useful will be mutant alleles that are dominant, but conditionally functional. A single mutation may provide both the dominant and conditional-growth phenotype. However, utilizing the existing collection of temperature sensitive alleles, mutagenesis with subsequent selection for a dominant allele may provide more mutational opportunities for obtaining the necessary dominant conditional alleles. There is precedent for such additive effects of mutations on the protein phenotype (T. Alber, 1989, *Ann. rev. Biochem.* 58:765-798) as well as evidence to suggest that heat-sensitive mutations, which generally affect internal residues (Hecht et al., 1983, *Proc. Natl. Acad. Sci. USA* 80:2676-2680), will occur at different locations in the protein different than dominant mutations, one type of which will affect protein-protein interactions, which are more likely on the protein surface.

The use of dominant conditional double mutants may have an additional advantage, since the hypersensitivity phenotypes may remain the same in the double mutant as in the single conditional mutant allele. In this case, a merodiploid carrying two copies of the target gene - one wild type, and one carrying the dominant conditional doubly

mutant gene - would provide a sophisticated screening strain (see Figure 18). The screen would rely on the hypersensitivity of the dominant protein to inhibitor compounds. Under conditions of the dominant protein's function, cells will not grow, while inhibition of the dominant protein will allow cell growth. The temperature sensitive allele provides a basis for hypersensitivity of the dominant protein, relative to the wild type protein.

c. Hypersensitive mutant alleles -

Additional mutants that display more pronounced hypersensitivities than the original conditional lethal mutants can be sought. Selection or screening procedures are based on the initial secondary phenotype profiles. These new highly hypersensitive alleles need not have a conditional growth defect other than that observed in the presence of the toxic agent or inhibitor. Such highly hypersensitive alleles provide strong target specificity, and high sensitivity to weak inhibitors. Such hypersensitive alleles can readily be adapted for screens with natural products, and with synthetic or combinatorial libraries of compounds in traditional screen formats.

d. Compound Binding and Molecular Based Assays and Screens

As indicated above, knowledge and possession of a sequence encoding an essential gene also provides knowledge and possession of the encoded product. The sequence of the gene product is provided due to the known genetic code. In addition, possession of a nucleic acid sequence encoding a

polypeptide provides the polypeptide, since the polypeptide can be readily produced by routine methods by expressing the corresponding coding sequence in any of a variety of expression systems suitable for expressing procaryotic genes, and isolating the resulting product. The identity of the isolated polypeptide can be confirmed by routine amino acid sequencing methods.

Alternatively, once the identity of a polypeptide is known, and an assay for the presence of the polypeptide is determined, the polypeptide can generally be isolated from natural sources, without the necessity for a recombinant coding sequence. Such assays include those based on antibody binding, enzymatic activity, and competitive binding of substrate analogs or other compounds. Consequently, this invention provides purified, enriched, or isolated products of the identified essential genes, which may be produced from recombinant coding sequences or by purification from cells naturally expressing the gene.

For use of binding assays in screening for compounds active on a specific polypeptide, it is generally preferred that the binding be at a substrate binding site, or at a binding site for an allosteric modulator, or at another site which alters the relevant biological activity of the molecule. However, simple detection of binding is often useful as a preliminary indicator of an active compound; the initial indication should then be confirmed by other verification methods.

Binding assays can be provided in a variety of different formats. These can include, for example, formats which involve direct determination of the amount of bound molecule, either while bound or after release; formats
5 involving indirect detection of binding, such as by determination of a change in a relevant activity, and formats which involve competitive binding. In addition, one or more components of the assay may be immobilized to a support, though in other assays, the assays are performed in
10 solution. Further, often binding assays can be performed using only a portion of a polypeptide which includes the relevant binding site. Such fragments can be constructed, for example, by expressing a gene fragment which includes the sequence coding for a particular polypeptide fragment
15 and isolating the polypeptide fragment, though other methods known to those skilled in the art can also be used. Thus, essential genes identified herein provide polypeptides which can be utilized in such binding assays. Those skilled in the art can readily determine the suitable polypeptides,
20 appropriate binding conditions, and appropriate detection methods.

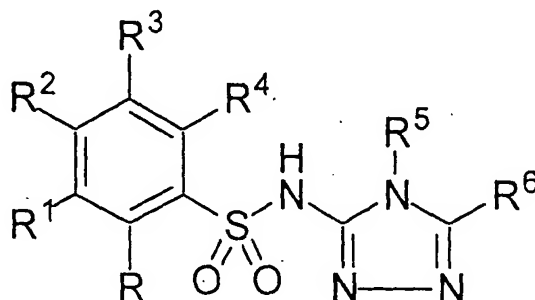
Provision of a purified, enriched, or isolated polypeptide product of an essential gene can also allow use of a molecular based (i.e., biochemical) method for
25 screening or for assays of the amount of the polypeptide or activity present in a sample. Once the biological activities of such a polypeptide are identified, one or more of those activities can form the basis of an assay for the

presence of active molecules of that polypeptide. Such assays can be used in a variety of ways, for example, in screens to identify compounds which alter the level of activity of the polypeptide, in assays to evaluate the sensitivity of the polypeptide to a particular compound, and in assays to quantify the concentration of the polypeptide in a sample.

10. Antibacterial Compounds Identified by Hypersensitive Mutant Screening

Using the genetic potentiation screening methods described above, a number of compounds have been identified which inhibit growth of *S. aureus* cell. These compounds were identified as having activity on the NT94 mutant described above, and so illustrate the effectiveness of the claimed screening methods. These results further illustrate that the genes identified by the temperature sensitive mutants are effective targets for antibacterial agents. The identified compounds have related structures, as shown in Figs. 19A-D

These compounds can be generally described by the structure shown below:



in which

R, R¹, R², and R³ are independently H, alkyl (C₁-C₅), or halogen;

5 R⁴ is H, alkyl (C₁-C₅), halogen, SH, or S-alkyl (C₁-C₃);

R⁵ is H, alkyl (C¹-C⁵), or aryl (C₆-C₁₀);

R⁶ is CH₂NH₂, alkyl (C₁-C₄), 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, or aryl (C₆-C₁₀);

10 or

R⁵ and R⁶ together are -C(R⁷)=C(R⁸)-C(R⁹)=C(R¹⁰)-, -N=C(R⁸)-C(R⁹)=C(R¹⁰)-, -C(R⁷)=N-C(R⁹)=C(R¹⁰)-, -C(R⁷)=C(R⁸)-N=C(R¹⁰)-, or -C(R⁷)=C(R⁸)-C(R⁹)=N-;

in which

15 R⁷, R⁸, R⁹, and R¹⁰ are independently H, alkyl (C₁-C₅), halogen, fluoroalkyl (C₁-C₅);

or

R⁷ and R⁸ together are -CH=CH-CH=CH-.

20 Thus, the invention includes antibacterial compositions containing the described compounds, and the use of such compositions in methods for inhibiting the growth of bacteria and methods for treating a bacterial infection in an animal.

25 V. Description of Compound Screening Sources and Sub-structure Search Method

The methods of this invention are suitable and useful for screening a variety of sources for possible activity as inhibitors. For example, compound libraries can be screened, such as natural product libraries,

combinatorial libraries, or other small molecule libraries.

In addition, compounds from commercial sources can be tested, this testing is particularly appropriate for commercially available analogs of identified inhibitors of particular bacterial genes.

Compounds with identified structures from commercial sources can be efficiently screened for activity against a particular target by first restricting the compounds to be screened to those with preferred structural characteristics. As an example, compounds with structural characteristics causing high gross toxicity can be excluded.

Similarly, once a number of inhibitors of a specific target have been found, a sub-library may be generated consisting of compounds which have structural features in common with the identified inhibitors. In order to expedite this effort, the ISIS computer program (MDL Information Systems, Inc.) is suitable to perform a 2D-substructure search of the Available Chemicals Directory database (MDL Information Systems, Inc.). This database contains structural and ordering information on approximately 175,000 commercially available chemical compounds. Other publicly accessible chemical databases may similarly be used.

VI. In vivo modeling: Gross Toxicity

Gross acute toxicity of an identified inhibitor of a specific gene target may be assessed in a mouse model. The inhibitor is administered at a range of doses, including high doses, (typically 0 - 100 mg/kg, but preferably to at least 100 times the expected therapeutic dose)

subcutaneously or orally, as appropriate, to healthy mice. The mice are observed for 3-10 days. In the same way, a combination of such an inhibitor with any additional therapeutic components is tested for possible acute toxicity.

VII. Pharmaceutical Compositions and Modes of Administration

The particular compound that is an antibacterial agent can be administered to a patient either by itself, or in combination with another antibacterial agent, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s). A combination of an inhibitor of a particular gene with another antibacterial agent can be of at least two different types. In one, a quantity of an inhibitor is combined with a quantity of the other antibacterial agent in a mixture, e.g., in a solution or powder mixture. In such mixtures, the relative quantities of the inhibitor and the other antibacterial agent may be varied as appropriate for the specific combination and expected treatment. In a second type of combination an inhibitor and another antibacterial agent can be covalently linked in such manner that the linked molecule can be cleaved within the cell. However, the term "in combination" can also refer to other possibilities, including serial administration of an inhibitor and another antibacterial agent. In addition, an inhibitor and/or another antibacterial agent may be administered in pro-drug forms, i.e. the compound is administered in a form which is

modified within the cell to produce the functional form. In treating a patient exhibiting a disorder of interest, a therapeutically effective amount of an agent or agents such as these is administered. A therapeutically effective dose
5 refers to that amount of the compound(s) that results in amelioration of symptoms or a prolongation of survival in a patient, and may include elimination of a microbial infection.

Toxicity and therapeutic efficacy of such com-
10 pounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and
15 therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50} . Compounds which exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The
20 dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. It is preferable that the
25 therapeutic serum concentration of an efflux pump inhibitor should be in the range of 0.1-100 $\mu\text{g/ml}$.

For any compound used in the method of the invention, the therapeutically effective dose can be estimated

initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} as determined in cell culture. Such information can be used to
5 more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by HPLC.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See, e.g., Fingl et al., in THE
10 PHARMACOLOGICAL BASIS OF THERAPEUTICS, 1975, Ch. 1 p. 1). It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunctions. Conversely, the attending physician would also know to
15 adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the
20 age, body weight, and response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

Depending on the specific infection being treated, such agents may be formulated and administered systemically
25 or locally. Techniques for formulation and administration may be found in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co., Easton, PA (1990). Suitable routes may include oral, rectal, transdermal, vaginal,

transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, 5 or intraocular injections, just to name a few.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For such 10 transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Use of pharmaceutically acceptable carriers to formulate the compounds herein disclosed for the practice of 15 the invention into dosages suitable for systemic administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, the compositions of the present invention, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection. The 20 compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art, into dosages suitable for oral administration. Such carriers enable the compounds of the invention to be formulated as tablets, 25 pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers including excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions. The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension,

such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores.

Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings.

For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can

5 contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid

10 paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added.

VIII. Use of Gene Sequences as Probes and Primers

In addition to the use of the growth conditional

15 mutant strains as described above, DNA sequences derived from the identified genes are also useful as probes to identify the presence of bacteria having the particular gene or, under suitable conditions, a homologous gene. Similarly, such probes are useful as reagents to identify

20 DNA chains which contain a sequence corresponding to the probe, such as for identifying clones having a recombinant DNA insert (such as in a plasmid). For identifying the presence of a particular DNA sequence or bacterium having that sequence it is preferable that a probe is used which

25 will uniquely hybridize with that sequence. This can be accomplished, for example, by selecting probe sequences from variable regions, using hybridization conditions of suitably high stringency, and using a sufficiently long probe (but

still short enough for convenient preparation and manipulation. Preferably, such probes are greater than 10 nucleotides in length, and more preferably greater than 15 nucleotides in length. In some cases, it is preferable that
5 a probe be greater than 25 nucleotides in length. Those skilled in the art understand how to select the length and sequence of such probes to achieve specific hybridization. In addition, probes based on the specific genes and sequences identified herein can be used to identify the
10 presence of homologous sequences (from homologous genes). For such purposes it is preferable to select probe sequences from portions of the gene which are not highly variable between homologous genes. In addition, the stringency of the hybridization conditions can be reduced to allow a low
15 level of base mismatch.

As mentioned above, similar sequences are also useful as primers for PCR. Such primers are useful as reagents to amplify the number of copies of one of the identified genes or of a homologous gene. As with probes,
20 it is preferable that the primers specifically hybridize with the corresponding sequence associated with one of the genes corresponding to SEQ ID NO. 1-105. Those skilled in the art understand how to select and utilize such primers.

25 The embodiments herein described are not meant to be limiting to the invention. Those of skill in the art will appreciate the invention may be practiced by using any of the specified genes or homologous genes, for uses and by

methods other than those specifically discussed, all within the breadth of the claims.

Other embodiments are within the following claims.